EXHIBIT A

FILED
2005 FEB -4 PM 3:36
WOODBURY COUNTY
CLERK OF DISTRICT COURT
BY _____ CLERK DESIGNEE

IN THE IOWA DISTRICT COURT FOR WOODBURY COUNTY

THE STATE OF IOWA, Plaintiff, vs. TROY DAVID KUNKEL, Defendant.	SRCR062687 ORDER RE: PROBATION REVOCATION HEARING

On February 4, 2005, the State's Application for Probation Revocation comes on for hearing. The State appears by Assistant Woodbury County Attorney Jill Pitsenbarger and the defendant appears in person and with his attorney Peter Monzel.

The defendant admits, by his signature below, that he violated his conditions of probation, as follows: Condition Number 1 – Obey all laws, by committing the criminal offenses of Absence from Custody and Contempt of Court; Special Condition Number 7 – Successfully complete the Residential Treatment Facility, by absconding from the Residential Treatment Facility; Condition Number 5 – Obtain prior permission before changing residency, by failing to obtain prior permission before changing residency; and, Condition Number 9 – Abstain from alcohol and illegal drugs, by using marijuana.

The parties agree that the defendant's probation shall be revoked and a 365-day jail sentence shall be imposed. The parties agree that the defendant shall be granted work search release and/or work release as verified by the Woodbury County Jail, but that the total amount of release per week shall not exceed 40 hours.

and/or treatment release

Defense Attorney

County Attorney

Defendant

IN THE IOWA DISTRICT COURT

FOR Woodbury **COUNTY**

FILE STAMP USE ONLY

FILED

'05 FEB -8 P1:47

CRAIG JORGENSEN
CLERK OF DISTRICT COURT

BY _____ CLERK DESIGNEE

State
Plaintiff/Petitioner

Attorney

vs.

Troy Kunkel
Defendant/Respondent

Attorney

No. SRCR062687

- ☐ Equity
- ☒ Criminal
- ☐ Law
- ☐ _____
- ☐ Calendar Entry
- ☒ Order
- ☐ Ruling
- ☐ Judgment

Now on this 8 day of February, 2005, the costs associated with this probation violation proceeding are taxed to the defendant, including any fees and expenses of court appointed counsel, if any.

return to
Clerk
SRCR062687

Copies mailed/delivered to:
Co atty P Mayer DCS

on 2-9, 2005, BY T Kruger
Clerk of District Court

BY THE COURT:
JOHN D ACKERMAN
JUDGE, 3rd JUDICIAL DISTRICT

HEARING HELD Yes No
If Yes: Contested Uncontested

RPC-1421-02

ENDORSED ORDER

The Court finds that the defendant has violated his conditions of probation pursuant to Iowa Code Section 908.11 as follows: Condition Number 1 – Obey all laws, by committing the criminal offenses of Absence from Custody and Contempt of Court; Special Condition Number 7 – Successfully complete the Residential Treatment Facility, by absconding from the Residential Treatment Facility; Condition Number 5 – Obtain prior permission before changing residency, by failing to obtain prior permission to change residency; and, Condition Number 9 – Abstain from alcohol and illegal drugs, by using marijuana.

The Court therefore revokes the defendant's period of probation and sentences the defendant to serve 365 days in the Woodbury County Jail with work search release and/or work release as verified by the Woodbury County Jail, but that the total amount of release per week shall not exceed 40 hours.

And/or treatment Release

Mittimus shall issue forthwith. Clerk to notify the parties.

Judge, 3rd Judicial District of Iowa

Asst. Atty. P.Monzel taken

2-4-05 SL

mitt issd 2-4-05

EXHIBIT B

IN THE IOWA DISTRICT COURT FOR WOODBURY COUNTY

STATE OF IOWA,
Plaintiff,

FILED

SRCR062687

TROY KUNKEL,
Defendant.

'04 MAY 13 A9:32

CRAIG HOENSEN
CLERK DISTRICT COURT

WRITTEN PLEA OF GUILTY AND WAIVER OF RIGHTS
CHILD ENDANGERMENT IN VIOLATION OF IOWA CODE §726.6(6)

COMES NOW, the above-named Defendant and states to the Court:

My attorney is the State of Iowa Public Defender's Office, 600 Benson Building, Sioux City IA, with whom I am satisfied and by whom I have been advised of my constitutional and statutory rights, and who I authorize to act on my behalf.

I have told my attorney all the facts and surrounding circumstances as known to me concerning the matters in the trial information and I believe that my attorney is fully informed as to all such matters. My attorney has informed, counseled, and advised me at length as to the nature and cause of each accusation against me as set forth in the trial information, and he has advised me as to any possible defense I might have in this case. I believe that my attorney has done all that can be done to counsel and assist me. There is nothing about the proceedings in this case which I do not understand fully. I am not now under the influence of any stimulant, drugs, or alcohol. I am not impaired in any way at this time so to affect my understanding of the proceedings herein.

ADVISORY OF ELEMENTS

I understand that in order to establish my guilt of the crime of child endangerment, that the State of Iowa would have to prove each and every of the following elements beyond a reasonable doubt:

That I was left with the temporary custody of a child, and that I acted in a manner that created a substantial risk of injury to the child.

ADVISORY OF RIGHTS

I have been advised and know that I may continue in my present plea of not guilty and have a trial. If I insist on my right to have a trial, I have certain important rights. I understand that if I change my plea to guilty, that I will give up all of the following rights:

a. I will be entitled to a speedy and public trial by a jury whose verdict must be unanimous in order to convict me;
b. That I will be presumed to be innocent until such time, if ever, that I am proven to be guilty, and the jury would be instructed accordingly;
c. That my guilt of each and every element must be proven beyond a reasonable doubt by competent evidence, and the jury would be instructed accordingly;
d. That I have a privilege against self-incrimination [my right to remain silent]. At a trial, I would have the right to testify or not as I desire, and my not testifying could not be used against me.
e. That I would confront my accusers and my attorney could cross-examine the witnesses called against me, and I would be present during all stages of the proceedings and the trial;
f. That I could subpoena witnesses on by own behalf, and require their presence and testimony in court.

I understand that my pleading guilty, I waive any right to a trial and that there will be no trial of any kind. My plea of guilty establishes my guilt beyond a reasonable doubt, so there is no need or right to a trial.

I understand that a criminal conviction, deferred judgment, or deferred sentence may affect my status under federal immigration laws.

ADVISORY OF DISCRETION OF COURT

I acknowledge that I am pleading guilty as my own voluntary and informed act because I am, in fact, guilty. I am not pleading guilty because of any threats of severe sentence or additional prosecutions or any other promises or threats. It is my understanding that the Judge may accept or reject the agreement between the parties which is indicated on the proposed sentencing order attached hereto. If the Judge accepts the terms and conditions set forth on said proposed sentencing order, the Judge will sign same. If the Judge does not accept anything about said proposed sentencing order, then the Judge will not sign same, and I will be permitted to withdraw

this plea of guilty and it can never be used against me. The decision as to an appropriate sentence is entirely within the sound discretion of the Court on the basis of the totality of the circumstances.

ADVISORY OF SENTENCE OPTIONS

I know that the maximum sentence as provided by law for this offense is a jail term not to exceed one year or a prison term not to exceed two years. A fine is required of at least $500. All fines are enhanced by a 30% surcharge in addition to the fine amount.

The Court is not required to impose any minimum sentence.

PLEA OF GUILTY

Rule 8 of the Iowa Rules of Criminal Procedure requires the Court to address me directly. I hereby request and approve the Court, in its discretion, to waive the provisions of Rule 8.

I know that by executing this Written Plea of Guilty and Waiver of Rights, I admit that I committed the elements of this charge of child endangerment to which I am pleading guilty, and that I may lose my liberty because of this plea of guilty.

What I actually did in Woodbury County, Iowa, on or about the date stated in the trial information was: **During a time when I was left with the temporary custody of a child, and that I acted in a manner that created a substantial risk of injury to the child.**

This is a bargained plea and is based on the aforementioned sentencing order being accepted by the Court. At the time of this signing, no one guaranteed to me any specific sentence other that what is agreed in the bargain.

KNOWING AND UNDERSTANDING ALL OF MY RIGHTS AND HAVING HAD THEM FULLY EXPLAINED TO ME, I DESIRE TO CHANGE MY PLEA TO GUILTY AS SET OUT BELOW:

I hereby enter my plea of guilty to the crime of child endangerment in violation of

of Iowa Code 726.6(6).

SENTENCING, WAIVER OF RIGHT TO MOVE IN ARREST OF JUDGMENT, WAIVER OF RIGHT TO DELAY

I request the Court to pronounce the judgment and sentence now and without delay at any place in the Third Judicial District of the State of Iowa.

If the Court wishes further pre-sentence investigation, the Court may take such additional time as the court requires.

I understand that I will be fined and will be required to pay the court costs herein, and that unless I pay same as required by the Court, that I may be held in contempt of court and lose my liberty.

I know that any challenge to a plea of guilty, based on defects or errors in the plea proceedings, must be raised in a Motion in Arrest of Judgment and that failure to raise such challenges shall preclude the right to assert such claims on appeal. Such a Motion must be filed within 45 days after the entry of this plea of guilty, but at least 5 days prior to the sentencing. Because I am not aware of any defects or errors which could be raised by such a Motion in Arrest of Judgment, and because I wish to be sentenced now, I hereby waive my right to a Motion in Arrest of Judgment.

I know that I have a right to a delay of at least 15 days between the date of the entry of my plea of guilty herein until the date for sentencing. Because I have no reason for the delay, and I am waiving my right to move in arrest of judgment, and because I wish to be sentenced now, I hereby waive my right to delay of time until sentencing.

APPEAL

I know that I have a right to appeal under Iowa Rule of Criminal Procedure 2.23(3)(e). If I am unable to pay the cost of appeal, I have the right to apply to the court for the appointment of counsel and the furnishing of a transcript of the evidence as provided in Iowa Code sections 814.9 and 814.11. I know that the filing of this Notice of Appeal is jurisdictional and that failure to timely file a Notice of Appeal within the time

and in the manner specified in Iowa Rule of Appellate Procedure 6.101 precludes my right to appeal.

CLOSING

I hereby waive reporting of these proceedings.

I HAVE READ ALL OF THE ABOVE AND UNDERSTAND ALL OF IT AND THE CONSEQUENCES OF THIS GUILTY PLEA, AND I REPRESENT TO THE COURT UNDER PENALTY OF PERJURY THAT MY PLEA OF GUILTY IS INTELLIGENTLY AND VOLUNTARILY MADE BY ME.

DEFENDANT

I, Michael K. Williams, a practicing attorney in the State of Iowa, state that I represent the defendant, that I have advised the Defendant at length and in detail regarding all of the Defendant's rights and that in my opinion the Defendant has full knowledge of those rights. In my opinion, there is a factual basis for the Defendant's plea of guilty and that the Defendant fully understands the consequences of this plea.

MICHAEL K. WILLIAMS
ATTORNEY FOR THE DEFENDANT

IN THE IOWA DISTRICT COURT FOR Woodbury COUNTY

FILED

'04 MAY 13 A9:32

CRA. J. JENSEN
CLERK OF DISTRICT COURT
BY _____ CLERK DESIGNEE

THE STATE OF IOWA,

Plaintiff,

vs.

TROY KUWKEL,

Defendant.

CRIMINAL NO. SRCR062687

ORDER

On May 10, 2004, the State appears by Jill Pitsenbarger and the defendant appears in person and by the defendant's attorney, Mike Williams.

Defendant pleads guilty (writing) to the crime of Child Endangerment in violation of Iowa Code Section 726.6(6). Plea accepted. Acceptance of plea agreement, if any, reserved to sentencing judge

It is ordered that:

1. June 30, 2004 @ 10:00 a.m. is fixed as the time for sentencing in this case.

2. A presentence investigation shall be made by the Third Judicial District Department of Correctional Services. Copies of the presentence investigation report shall be furnished to the County Attorney and the defendant's attorney. A copy of the presentence investigation report shall be furnished to this court at least three days prior to the date fixed for sentencing.

3. Bail: Same

4. The clerk of court shall mail or deliver copies of this order to counsel of record and the Third Judicial District Department of Correctional Services.

DUANE E HOFFMEYER Judge of the
Third Judicial District of Iowa

cc: COA
PRob
DCS
5-17-04

EXHIBIT C

IN THE UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF IOWA

WESTERN DIVISION

THE SECURITY NATIONAL BANK OF SIOUX CITY, IOWA, as conservator for J.M.K., a Minor, Plaintiff, vs. ABBOTT LABORATORIES, Defendant.	NO. 5:11-cv-0417-DEO VIDEOTAPED DEPOSITION OF TROY KUNKEL

The Videotaped Deposition of TROY KUNKEL taken at 1128 Historic 4th Street, Sioux City, Iowa, on the 6th day of July, 2012, commencing at 8:02 a.m.

really just like a one station person. Especially in restaurants.

Q. Okay. Okay. Now I want to find out a little bit more about some more of your family. There have been people that have been talked about in this lawsuit and some other people that you're related to. My understanding is that you have a brother and two sisters?

A. Yes.

Q. Okay. So your brother is Adam?

A. Yes.

Q. And how old is Adam?

A. Forty.

Q. And where does he live?

A. Here in Sioux City.

Q. Okay. And you have a sister Penny?

A. Yes.

Q. And I didn't catch the last name yesterday, so what is her last name?

A. Maiden or married?

Q. Her current last name.

A. Fannon, F-A-N-N-O-N.

Q. Okay. And how old is she?

A. I'm 29. Thirty-four.

Q. And is she here?

A. Dixon, Nebraska.

Q. And then is there a Stacy?

A. Yeah, but there is no use even asking me about her. I don't -- that --

Q. You don't --

A. I have no idea. I don't even --

Q. When was the last time you heard from her?

A. When my mom died in '09.

Q. So you don't know what her last name is or where she lives?

A. I -- She could have five different last names, for all I know. Last I knew it was Pasavak (phonetic).

Q. Okay. And your parents names, your father's name is James?

A. Yes.

Q. And your mom's name was Deborah?

A. Yes.

Q. And you said that she passed away in 2009. And your father is still alive, correct?

A. Yes.

Q. And do you know back in 2008, about the time that Jeanine was born -- or at the time that Jeanine was born, do you know if your father was employed?

A. No, he wasn't. He hasn't been able to work since he basically retired after Wells' Blue Bunny. He was on social security. He fell and got hurt.

Q. How about your mom at the time, was she --

A. My mom wasn't able to be employed for like the last 10, 12 years because of health problems.

Q. And your dad right now is incarcerated?

A. Yes.

Q. Is it in the State of Iowa?

A. No.

Q. Do you know what state it is in, or is it federal?

A. It is a federal facility. He is in Minnesota. Rochester Medical.

Q. And back in 2008, when did you -- it sounds like at least one of your grandparents were alive at the time, Rose

Kerns?

A. Yes.

Q. Are any of your other grandparents alive?

A. No.

Q. Now do you use recreational drugs like marijuana?

A. Yes.

Q. Any others?

A. I have in the past.

Q. Which ones?

MR. RATHKE: Ever or --

MR. SCANNAPIECO: Ever.

MR. RATHKE: In a given time frame.

MR. SCANNAPIECO: In the past.

MR. RATHKE: I'm going to object to the question in that it's overbroad.

MR. SCANNAPIECO: Okay.

MR. RATHKE: Go ahead and answer. I guess he wants to know every drug you have ever taken.

A. Can I ask why that's -- I -- I'm just asking why -- why it is important.

Q. Do you have some problems with --

you know, if you see needles, or things like that --

A. Well, I don't have problems. I am a diabetic, so I can't really have a problem with needles; but I mean, yes, I have used meth in the past. I mean if you got my file, you know my father was incarcerated for distributing meth.

Q. There's not like a -- just so you know, there is not like a master file on you.

A. I am -- it's nothing that I'm trying to hide or lie about. I am very open in my past and my drug use and I have nothing to hide about it. I don't want something that I did 15 years ago to be used against me at any point, or because --

MR. RATHKE: Or against Jeanine.

A. -- or against Jeanine, yeah, because really that -- what I did 15 years ago doesn't, you know, really have nothing to do with her today. But for the record, yes, I have used recreational drugs.

Q. Okay. And so marijuana. Do you

use that off and on up until today?

A. I'm currently sober. But, yes, I was using it on and off up until recently.

Q. Were you using it back in 2008?

A. No, I wasn't, actually. It wasn't -- I was actually sober for several years until after the Omaha -- it wasn't until we came back from Omaha, and everything, I was -- right before we came back, I made a trip up and -- to get away. And I was really stressed, you know. But, like I said, I had been clean for several years up until that point.

Q. So when was the last time you used any other drugs other than marijuana, maybe --

A. Gosh, over a year. It has been over a year.

MR. RATHKE: Other than marijuana.

A. Yeah. I haven't used nothing other than marijuana over a year now. I don't --

Q. Okay.

A. I don't really drink. I don't --

Q. When was the last time you used

meth?

A. Over a year ago.

Q. So sometime in 2011?

A. Uh-huh (Yes). Well, when --

Q. Did you ever use it in 2010?

A. Not -- I don't recall. I --

Q. How about after you came back from Omaha, did you use it then?

A. No. It was just the marijuana.

Q. Okay. Do you smoke?

A. Cigarettes, yes.

Q. And about how much do you smoke per day?

A. Maybe half a pack, if that.

Q. How long have you smoked?

A. Since I was 10. Nine, turned 10.

Q. So about the early '90s?

A. (Moves head in an affirmative manner.)

Q. Okay. Have you always smoked about a half a pack a day?

A. Have I always? No. No.

Q. That kind of laugh suggests more to me.

A. It depends on the day, I guess,

but on average, half a pack a day.

Q. Sometimes more? Sometimes less?

A. Yes.

Q. You were smoking during the pregnancy of the twins?

A. Yes.

Q. Do you ever drink?

A. Occasionally. Very, very, very, very occasionally.

Q. More than once a month?

A. Not even that. Not even that. Maybe once a year.

Q. Okay. And has that always been the case, or were there times that --

A. I was young and dumb once. When I was younger, I drank a lot more, but the last four years I have been dealing with diabetes, and so it is kind of hard to really, you know, do much else with my diabetes.

Q. Okay.

A. Even doing a shot, you know, going to the bar and doing one shot affects my diabetes, so --

Q. So you have diabetes. Do you have

2nd. It is on both pages. Do you see that?

MR. RATHKE: I'd feel a lot more comfortable if there is some better indication of when -- of when this sign out occurred. I see what you're saying, but --

MR. SCANNAPIECO: Okay.

MR. RATHKE: -- my experience with medical records is that sometimes dates get on there for odd reasons and may not correlate to the date of the event, but whatever.

Q. Okay. Do you ever recall having to check out of the hospital and then come back in --

A. Yes.

Q. -- on the same day?

A. Yes.

Q. Okay. And do you recall what your symptoms were at the time that that happened?

A. If this is right, this is when I went to pee and my blood sugar was 700 almost.

Q. Okay.

A. Or was around 700. Like I said, I was going ketoacidosis.

Q. Okay. And when you were going into ketoacidosis, before that had you been nauseous?

A. Vomiting, yeah.

Q. Okay.

A. Losing weight. Couldn't eat. Couldn't hold my food down.

Q. Okay. And were you at the -- were you going through this at home? Like where was this happening?

A. At home. This -- this was the early stages of my diabetes. This was when I was just like learning how to be a diabetic.

Q. Okay.

A. I had a lot of problems with it at first.

Q. Okay.

A. So I -- I had -- I -- I mean I'm sorry I'm not recalling. I have got so many hospitalizations and doctor visits and doctors over my diabetes, I'm trying

to really cipher through six years of doctors and medical and being admitted into the hospital for it and -- but I can't recall dates, or nothing like that, to be specific, no.

Q. Okay. Now during that -- your time at St. Luke's for when you said you were learning about your condition and when you were in there, checked out and came back, do you recall them ever running any drug tests on you?

A. Probably. They do every time. They take pee.

Q. Yeah. Okay.

A. And there was probably marijuana in there.

Q. What did you say?

A. There was probably marijuana in there.

Q. Okay. So was that -- were you -- was that one of the times that you were -- around the time you were using it, around April of 2008?

A. If it was in there, then, yeah. I -- I mean I don't recall specific use,

or nothing, you know. But it could have been -- because, like I said, around '08 and before that, I was -- I wasn't doing really nothing. It could have been a -- a slipup. I don't --

MR. RATHKE: Okay. Do you have a document to show him on that? I mean the insinuation is they flunked -- that marijuana was found.

A. I mean they never told me anything like that. I mean --

Q. Okay.

A. If the doctor -- because every time I go in for a three-month checkup, the doctor does a UA.

Q. Can you please put a --

A. He has never -- you know, they've never come to me and said, well, hey, there's marijuana in your system, or nothing like that, you know.

Q. Okay.

A. I mean they know my past. And I have been very open with my doctors, so --

Q. Okay.

A. But, like I said, if there was

some in there, if I recall, it was at a time that it was very, very little in my life. Very -- I had a lot going on.

Q. All right. So let me --

A. I might have went to the bar one night with a bunch of friends to celebrate having kids and somebody popped out a joint, you know. That could be the case. But I -- I do know that during this whole beginning part of my diabetes, I wasn't --

Q. Okay.

A. -- actively --

* * * *

(Exhibit 20 was marked.)

* * * *

Q. Okay. So I'm handing you what's been marked as Deposition Exhibit No. 20. It's a two-page document that at the top says St. Luke's Regional Medical Center. The bottom says SLT 131 through 132. Okay? Have you seen this document before?

A. Never.

Q. Okay. Now if you look at the second page, there is a section there that says Other Tests. And then the first line

is Urine Drugs of Abuse. Do you see that?

A. Uh-huh (Yes).

Q. So then it has different -- it looks like different types of drugs, right? So like cocaine, none detected. Opiates, none detected. Do you see that?

A. Yes.

Q. And under cannabinoids, it says your patient is presumptive positive for this drug group.

A. Uh-huh (Yes).

Q. Did anybody talk to you about those results?

A. No.

Q. Did you remember taking a urine test when you went to the hospital --

A. I do.

Q. -- in early April?

A. I -- well, obviously -- I mean I don't remember it, no, but obviously it did happen.

Q. Okay.

A. But, like I said, I do recall 99 percent of the times I go see a doctor, or anything, they do -- that's one of the

ways they check certain things for diabetics, is through your urine. So I mean of course they're going to screen for everything else because of my history.

Q. Okay.

A. So I mean that's -- it is new to me that they're screening like that, but I'm not saying they didn't have a reason to.

Q. Okay.

A. Oh, the doctor that you are thinking of is Dr. Moreno. Not Dr. Morris.

Q. Dr. Moreno?

A. Yes.

Q. Okay.

A. Because he's -- he's the understudy, or whatever, or under doctor at Dr. Peterson's office over there at Midtowns Clinic on Outer Belt.

Q. Okay. Can you put --

A. You see Peterson. If Peterson is not in, you see Moreno.

Q. Now during those early hospitalizations that you had, did they

ever perform something like a lumbar puncture or anything like that on you, a spinal tap?

A. I have had one, but way back when I was younger, not through none of this, no.

Q. Okay.

A. Not that I recall.

Q. Do you recall if they did anything other than urine test? Did they do like a stool test or anything like that? Do you remember that?

A. I know they took all kinds of samples of stuff. Blood. They did some cultures where they put it in tobacco sauce looking bottles --

Q. Okay.

A. -- to see if it grows. I mean they did all kinds of tests, so you would have to get the records for that. I don't --

Q. Okay.

A. I've had so many tests done on me in the last four years, it's unreal.

Q. All right. So now let's move

ADDENDUM

I, Troy Kunkel, find the following corrections to my deposition given on July 6, 2012, in Case 5:11-cv-04017-DEO.

PAGE	LINE	CORRECTION

NO CORRECTIONS

If you make no corrections, please indicate "No Corrections."

Deponent's Signature

CERTIFICATE OF DEPONENT

I, Troy Kunkel, the undersigned deponent, hereby state under oath that I did read the foregoing pages of transcript; that any corrections I want to make the foregoing pages of transcript have been set out on the foregoing Addendum; and that I have indicated the correction itself and the page and line number of the correction, if any.

In witness whereof, I hereunto affix my signature this 21 day of August, 2012, before the undersigned Notary Public.

Deponent's Signature

I did witness the above signature on this 21 day of August, 2012, in the City of Sioux City, County of Woodbury, State of Iowa.

NOTARIAL SEAL IOWA
KRISTI J MULLINS
Commission Number 773857
My Commission Expires
July 13, 2015

Notary Public

My Commission Expires: July 13, 2015

ST.LUKE'S REGIONAL MEDICAL CENTER
Dept of Pathology and Clinical Lab Services
2720 Stone Park Blvd.
Sioux City, Iowa 51104

Name: KUNKEL,TROY D
Med Rec #: 17330138
Loc: X5A1 Rm: X511-P Sex: M
Acct #: 146174412 DOB: [redacted]
Admit Date: 04/03/2008
Disch Date: 04/04/2008

********** HEMATOLOGY - Cell Counts/Differentials **********

DAY:	0		
DATE:	04/02/08		
TIME:	2316	REF RANGE	UNITS
LOC:	EMR		
FOOTNOTE:	#1		
BASO ABSOLUTE	0.03	0.0-0.2	th/mm3

#1 DIFF TYPE - AUTOMATED DIFFERENTIAL

********** URINALYSIS Routine **********

DATE:	04/03/08		
TIME:	0121	REF RANGE	UNITS
COLOR	YELLOW		
CLARITY	CLEAR		
GLUCOSE	>=1000*	NEG	mg/dL
BILIRUBIN	NEG	NEG	
KETONES	TRACE*	NEG	mg/dL
SP GRAV	1.023	1.003-1.035	
BLOOD	NEG	NEG	
PH	5.0	5.0-9.0	
Protein	NEG	NEG	mG/dL
UROBIL	0.2	0.2	EU/dL
NITRITES	NEG	NEG	
LEUK EST	NEG	NEG	

********** CANCELLED TESTS **********

04/05/08	2100	CANCELLED: BEDSIDE GLUC MONITOR REASON:CANCELLED BY HIS
04/05/08	1600	CANCELLED: BEDSIDE GLUC MONITOR REASON:CANCELLED BY HIS
04/05/08	1100	CANCELLED: BEDSIDE GLUC MONITOR REASON:CANCELLED BY HIS
04/05/08	0630	CANCELLED: BEDSIDE GLUC MONITOR REASON:CANCELLED BY HIS
04/04/08	2100	CANCELLED: BEDSIDE GLUC MONITOR REASON:CANCELLED BY HIS
04/04/08	1600	CANCELLED: BEDSIDE GLUC MONITOR REASON:CANCELLED BY HIS
04/04/08	1100	CANCELLED: BEDSIDE GLUC MONITOR REASON:CANCELLED BY HIS

********** OTHER TESTS **********

04/02/08
2316 KETONES
10 mg/dL

04/03/08
0121 URINE DRUGS OF ABUSE
AMPHETAMINES URN
[NONDET]
NONE DETECTED
BARBITURATES URN
[NONDET]

<<RESULTS CONTINUED ON NEXT PAGE>>

LEGEND
H-High *-Abnormal
L-Low C-Critical

Name: KUNKEL,TROY D Phys: MARINO, Frank S
Loc: X5A1 Rm: X511-P Phys: PETERSON, PAUL D
Print Date: 04/05/2008 Page 3
INPATIENT FINAL-MED RECORD COPY CONTINUED

$$PAGE

OTHER TESTS

URINE DRUGS OF ABUSE <<CONTINUED FROM PREVIOUS PAGE>>
NONE DETECTED

BENZOS URN		
	[NONDET]	
	NONE DETECTED	
CANNABINOIDS URN		
	[NONDET]	
	*	Your patient is presumptive positive for this drug group. If you wish confirmation of this drug group, please contact the lab within three (3) days of the collection date. Specimens will then be forwarded for confirmation or quantitation if requested.
COCAINE URN		
	[NONDET]	
	NONE DETECTED	
OPIATES URINE		
	[NONDET]	
	NONE DETECTED	
U SPECIFIC GRAVITY		
	1.023	
URINE PH	5.0	

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EXHIBIT D

A comparative study of the effects of methamphetamine on memory in existing and recovering addicts from a South African population

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Memory is a complex of systems by which an organism registers, stores and retrieves exposure to an event or experience. Literature purports that methamphetamine users and dependents have been found to exhibits signs of memory impairment. The aim of the research was to establish the possible existence of significant differences in memory in current methamphetamine users, recovering methamphetamine users, and a matched drug naïve control group. Cognitive functioning was assessed via a neurocognitive test battery that examined the memory of 14 current methamphetamine users, 17 recovering methamphetamine addicts, and 18 drug naïve control participants who were matched according to the demographic variables of age, gender and educational status. The results indicated that recovering methamphetamine users experienced the greatest impairment in memory in comparison to both the control group and current users of methamphetamine. The current users of methamphetamine also experienced some impairment in memory functioning in visual acquisition and retention. The poor performance of the recovering addicts is explained by the juxtaposition of the stimulating and supplemental effect of methamphetamine as experienced by the current users versus the neurotransmitter depletion and structural changes in the brain experienced by the recovering addicts. The control group showed a superior performance since they did not suffer from the neurotoxic effects of methamphetamine.

Geheue is 'n komplekse sisteem wat 'n individu in staat stel om blootstelling aan 'n voorval of ervarings te registreer, stoor, behou en herroep. Leer- en geheueprobleme is van die mees algemene simptome van neurosielkundige uitvalle in neurologiese en psigiatriese pasiënte. Die literatuur dui aan dat metamfetamienafhanklike verbruikers tipies geheuedisfunksie ervaar. Die doel van die navorsing was om die moontlike voorkoms van verskille in geheuefunksie in huidige gebruikers van metamfetamien, rehabiliterende gebruikers, sowel as 'n kontrolegroep van dwelmmiddel-naïewe demografies-passende individue te bepaal. Uitvoerende funksie is gemeet met 'n neurokognitiewe toetsbattery wat die geheuefunksies van 14 huidige gebruikers van metamfetamien, 17 rehabiliterende metamfetamienverslaafde individue en 18 dwelmmiddel-naïewe deelnemers, gepas in terme van ouderdom, geslag en opvoedkundige status, bepaal het. Die resultate dui aan dat dat die rehabiliterende metamfetamiengebruikers die grootste geheueuitvalle getoon het in vergelyking met sowel die huidige gebruikers as die kontrolegroep. Die huidige metamfetamiengebruikers het ook matige geheueuitvalle getoon, spesifiek in visuele leer en retensie. Dit is moontlik dat die geheueuitvalle wat deur metamfetamiengebruikers ervaar word, verband hou met strukturele en funksionele verandering in die breingebiede wat met geheue geassosieer word, as gevolg van metamfetamienvergiftiging. Die swak prestasie van die rehabiliterende metamfetamienverslaafde persone in vergelyking met die huidige gebruikers word verduidelik in terme van die naasmekaarstelling van die stimulerende en aanvullende effek van metamfetamien soos ervaar deur die huidige gebruikers versus die neurotransmitteruitputting en strukturele breinveranderinge in die rehabiliterende individue. Die kontrolegroep het 'n beter resultaat getoon omdat hulle geen neurotoksiese effekte van metamfetamien gehad het nie.

Introduction

Methamphetamine is a highly addictive psycho-stimulant (Barr *et al*. 2006:301) that has become increasingly abused over the past several years for its euphoric effects (Hart *et al*. 2001:75). Twenty-nine million people consumed amphetamine-type stimulants in the late 1990s, a larger number than that of people using cocaine and opiates combined (World Health Organisation 2001:7).

Problem Statement

The need for scientific investigations regarding the effects of methamphetamine use is highlighted by the express worldwide and local resurgence of methamphetamine prevalence and abuse (Barr *et al.* 2006:301; Cape Town Drug Counselling Centre [CTDCC] 2005; Volkow *et al*. 2001a: 377).

South Africa has a dearth of information on the effects of methamphetamine on memory in its own population despite the prevalence of use locally (Plüddemann, Myers & Parry 2008:964). The focus of South African investigations into the methamphetamine epidemic often revolve around the social and economic correlates of this drug use, as well as its links to mental health problems and violence (Kapp 2008:193–194; Simbayi *et al.* 2006:291–300).

Limited studies exist that evaluate the effects of methamphetamine on current users, but virtually no research exists on simultaneous comparative studies of the effects of methamphetamine use in current and recovering users in comparison to each and a matched control group.

Additionally, of the studies that focus their research on the deleterious neurological and neurocognitive effects of methamphetamine, many are centred on these effects in early stage recovering or abstinent methamphetamine addicts (Ernst *et al*. 2000:1344–1349; Gonzalez, Bechara & Martin 2007:155–159; Johanson *et al*. 2006:327–338; Kalechstein, Newton & Green 2003:15–29; Salo *et al*. 2005:310–313; Sekine *et al*. 2001:1206–1214; Sung *et al*. 2007:28–35; Volkow *et al*. 2001a:377–382, 2001c:2015–2021). There are few studies that focus on the neurological and neurocognitive effects of methamphetamine in current users (McKetin & Mattick 1998:181–184; Simon *et al*. 2000:222–231). There are also no known studies that compare the cognitive functioning of recovering methamphetamine addicts and current users simultaneously, particularly in South Africa.

Background

Methamphetamine is known locally as 'crystal meth' or 'tik'. Users often feel dramatic increases in energy, alertness, sexual arousal, appetite and pleasure, increased self-confidence and grandiosity, an overall sense of well-being, and reduced appetite (Abadinsky 1997:120; Levinthal 2005:101; Nordahl, Salo & Leamon 2003:318; Yudko, Hall & McPherson 2003:55). Chronic doses may result in negative symptoms such as tremors, hyperflexia (muscle spasms), malnutrition, bruxism (teeth grinding), athetosis (strange muscle movements), agitation, restlessness, rage, insomnia, anxiety, hallucinations of formication (the sensation of insects crawling under the skin), and paranoia with the potential of severe amphetamine-induced psychosis (Anglin *et al.* 2000:139; Barr *et al.* 2006:302; Levinthal 2005:101; Yudko *et al*. 2003:55). Users may suffer from increased blood pressure, body temperature (hyperthermia), breathing rate as well as cardiac arrhythmia, stroke and potential cerebral convulsions and coma (Barr *et al.* 2006:303).

Studies of methamphetamine users have found evidence to suggest that the effects of methamphetamine use extend well beyond the interval of active use. International pre-clinical and clinical studies have found that methamphetamine abuse has been associated with residual negative effects noted in long-term neural damage in humans including a number of chemical, metabolic, neuronal and or physiological alterations (Sekine *et al*. 2001:1212; Tong *et al*. 2003:899; Volkow *et al*. 2001a:381; Volkow *et al*. 2001b:387). Observations of methamphetamine users have also led researchers to conclude that the observed deficits users exhibit, in areas such as abstract reasoning, planning, memory, attention, executive functioning and behavioural flexibility, may be as a direct result of methamphetamine's neurotoxicity (Barr *et al*. 2003:301; Nordahl *et al*. 2003:317, 322; Yücel, Lubman, Solowij & Brewer 2007:961).

Locally, methamphetamine has become a serious public health concern in the Western Cape. A 41.5% increase in methamphetamine as a primary drug of abuse has been noted from 2002 to 2006 in Cape Town (Plüddemann, Myers & Parry 2008:964). The startling increase of methamphetamine use in South Africa is further exacerbated by the fact that 80% of methamphetamine users in the Western Cape are under 21 years of age, according to the South African National Council on Alcoholism and Drug Dependence (SANCA) (Morris & Parry 2006:471). These figures indicate an express need for continued scientific investigations into the effects of methamphetamine.

Objectives

The core research objectives explored in this study are explained as follows:

- To address the scarcity of literature and quantitative research on methamphetamine and its role in South Africa – particularly in a neuropsychological context – while providing a template upon which further research can build.
- To establish the existence of potential cognitive impairment in the area of memory in a group of 14 addicts currently using methamphetamine compared to a group of 17 abstinent recovering methamphetamine addicts and a matched control group of 18 participants.

Significance of the study

Little to no information exists on the physiological and psychological effects of methamphetamine on cognition, particularly on the actual neuropsychological prognosis of local methamphetamine users both current and recovering. As such, this study, as one of a limited number of studies that comparatively studies both current and recovering methamphetamine users simultaneously, will contribute to the existing international literature base surrounding methamphetamine and its neuropsychological effects.

In doing so, this research will also aid in addressing the dearth in research on methamphetamine and its effects on

the South African population. This is particularly important in South Africa where methamphetamine abuse has become widespread. Additionally, the comparative nature of this study may also aid in determining why methamphetamine treatment programmes are plagued by high relapse and low retention rates (Copeland & Sorenson 2001:91). Moreover, this information may further assist in the determination and construction of more effective rehabilitation and treatment programmes, with the possibility of contributing to the development of a promising prevention programme.

Research method and design

Design

The research utilised an ex post facto quantitative and comparative study design. An ex post facto design was deemed most appropriate for this study which studied the effects of an illegal substance that is dangerous and harmful, precluding the use of an experimental design. Therefore, only those people who are already using the drug at their own discretion, their own usage method and their own quantity were approached. It is comparative and quantitative as the research wishes to compare three different groups with each other in order to ascertain where possible quantifiable differences might occur. The present research was also conducted in a positivistic research paradigm in that the object of the study is independent of the researcher.

Sampling

Inclusion criteria for the research were described as follows: 1) all participants were required to be fluent in English; 2) over 18 years of age and under 40 years of age; 3) have a Grade 12 matriculation certificate or equivalent; 4) be free from psychosis upon testing; 5) have no diagnosed acute Axis 1 mental disorder diagnosis (excluding drug-induced depression), or aneurysm, head injury with a loss of consciousness, a history of temporal lobe epilepsy, HIV+ status, multiple sclerosis, attention deficit disorder with or without hyperactivity, a learning disability, or any other neurological or medical condition known to affect cognitive status. Recovering addicts and the control sample were required to be free from alcohol and sedatives at least 24 hours before testing. All potential participants not meeting the criteria were not excluded.

A total of 14 current methamphetamine users (9 male users, 5 female users) were sampled all of whom had a minimum of one year regular methamphetamine use, and were determined to be either substance dependent or abusers according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR) (American Psychiatric Association [APA] 2000:197, 199). The currently using participants were obtained via 'word-of-mouth' from recovering methamphetamine users and acquaintances from the greater Johannesburg area in Gauteng as this is where the current users resided.

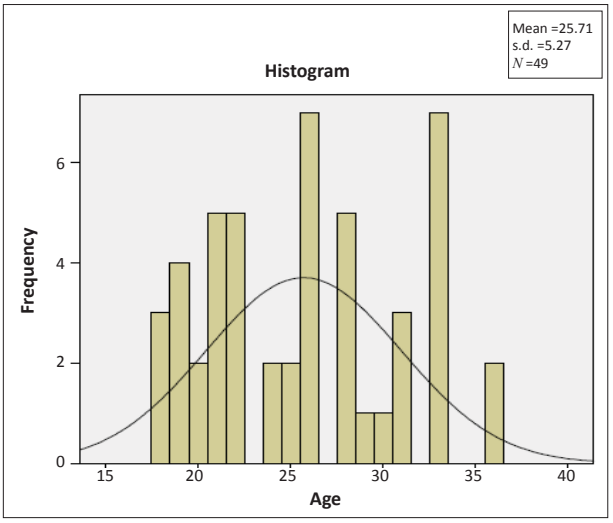
Seventeen recovering, treatment-seeking methamphetamine users (8 male users, 9 female users) were recruited from rehabilitation and treatment centres in Gauteng and the Western and Eastern Cape on recommendations from treatment professionals currently working within the treatment centres. These participants were required to have used methamphetamine regularly for a minimum of one year prior to seeking treatment, with a DSM-IV-TR (APA 2000:197, 199) diagnosis of substance abuse or dependence, and were required to be sober from all drugs and alcohol for a minimum of one month prior to this research's assessment. The sobriety of this group was ensured through urine screening as part of their inpatient status in the treatment facilities. Participants who were in outpatient programmes were asked to confirm their sobriety verbally and these assurances were assumed correct as they were still currently engaged in substance abuse treatment and rehabilitation.

The control group consisted of 18 participants (9 male users, 9 female users) who were found subsequent to the assessment of the two experimental groups through recommendations from peers according to the matching criteria and were required to be completely drug naïve. The control group was matched to the experimental groups according to age, gender and educational status.

No identifying information was required from the participants.

Neuropsychological Battery

Memory was assessed using the following neuropsychological tests: the Map Memory test, the Picture-Number test, and the



s.d., Standard Deviation

FIGURE 1: Histogram of Age Distribution for 2 Experimental Groups and 1 Control Group Combined.

TABLE 1: Gender Distribution of Participant Samples.

Group	*n*	Gender	
		Male	Female
Group 1 Current Users	14	9	5
Group 2 Recovering Addicts	17	8	9
Group 3 Control Group	18	9	9
Total	49	26	23

n, number of participants.

Auditory Number Span test from the Kit of Factor-Referenced Cognitive Tests (KFRCT) (Ekstrom *et al.* 1987:94, 102, 110).

The tests in the Kit of Factor-Reference Cognitive Tests (KFRCT) were developed in 1987 for the Educational Testing Service in Princeton, New Jersey and are based on factors identified by great names in psychology and psychometric testing such as Raymond Cattel, John Carroll, Thurstone, and J.P. Guilford (Ekstrom *et al*. 1987). The tests were designed for research purposes only, by the Office of Naval Research in the United States of America.

It is important to note that the reliability and validity of the measuring instruments in any study impact the generalisation capacity and appropriateness of the results obtained during the course of the research. The KFRCT (Ekstrom *et al*. 1987) consists of highly regarded researchers' tests and do not reflect reliability and validity coefficients in the testing manual. The current research therefore calculated the reliabilities of the following tests using the Cronbach's Alpha Test of Reliability. The psychometric properties of the tests are listed below.

The Map Memory Test

The Map Memory test is a two-part test of visual recall or recognition (Ekstrom *et al*. 1987:113). Each part requires a participant to 'study' a test sheet of 12 different maps for a period of four minutes. The participant is then presented with a testing page, which presents 12 maps of which some are the same and some are new. The participant is required to indicate whether they have (Y) or have not (N) seen the map on the previous study page. The scores from the two parts of the test are allocated, based on the number of correct identifications of previously seen and unseen maps and are added together to yield a single overall score.

The reliability for this test was determined during the course of the research using the Cronbach's Alpha Test of Reliability. This test produced internal consistency coefficients of also states that visual recognition 0.795 overall, which falls into the same bracket as other visual recognition tests. Lezak (1995) tests, such as The Map Memory Test from the KFRCT (Ekstrom *et al*. 1987:113), have reliability coefficients ranging from 0.7–0.8.

This test is similar to subtests from the Weschler Memory Scale, which has proven validity. Most of the participants in this study exhibited observational difficulty with visual memory and this test purports to assess visual recall and recognition, and as such the participants performed poorly on this test, ensuring the face validity of this test.

The Picture-Number Test

The Picture-Number Test is a visual learning (acquisition) and memory (retention) test in two parts (Ekstrom *et al*. 1987:94). In each part, the participant is presented with a page of 21 pictures of common items, which are paired with a two-digit number. The participants are given four minutes to 'study' this page after which they are instructed to turn to the 'test' sheet, which presents only the pictures in a different order than the study sheet. The participants are then required to fill in the corresponding number to match the picture in the three-minute time limit. Scores on this test are derived from the number of correct picture-number combinations that have been remembered and the two parts are added together to yield a single score.

This test showed internal consistency coefficients of 0.921 overall, with reliability coefficients of 0.887 for part one and 0.843 for part two. The test is similar to other paired-associate visual acquisition and learning tests as found in the Wechsler Memory Scale, which has proven validity, and thus the validity of this test is assumed.

The Auditory Number Span Test is a 'conventional digit span forwards test, which assesses storage and retrieval in short-term memory' (Ekstrom *et al*. 1987:101). Digit Span tests assess the limits of the participant's capacity for encoding and briefly retaining a series of numbers. This test has one condition of 24 digit series, yielding a single score, which determines the participant's ability to recall a number of distinct elements for instant reproduction (Ekstrom *et al*. 1987:102). The examiner reads out a digit series of varying lengths at the speed of one digit per second and, once the series has been read, the participant is allowed to write down what they can remember of the series.

This test produced internal consistency coefficients of 0.819 overall, which are high reliability coefficients. The Auditory Number Span Test is a conventional digit-span test that assesses immediate verbal retention by demonstrating an individual's ability to recall a number of distinct elements for immediate reproduction (Ekstrom *et al*. 1987:102). This digit-span test is similar to the one utilised in the Wechsler Memory Scale, which has good reported construct validity as a measure of verbal learning and memory (Larrabee, Kane & Schuck 1983:159).

Data collection method

The biographical questionnaire and neurocognitive test battery were administered in one hour to the participants in quiet surroundings that were both private and free from distraction. The biographical questionnaire served as both a screening process for inclusion and exclusion criteria, as well as a diagnostic tool to determine a DSM-IV diagnosis of substance abuse or dependence currently or previously.

This study was conducted across three provinces: Gauteng, the Eastern Cape and the Western Cape. Current methamphetamine users were interviewed and assessed in the privacy of their, or their friend's, home. Recovering methamphetamine addicts were interviewed and assessed at local rehabilitation centres (if inpatients), or public locations such as their homes or community centres (if outpatients). The control group was assessed and interviewed wherever was most favourable for them.

TABLE 2: Comparisons of Group 1 (Current Users) and Group 2 (Recovering Addicts) regarding their scores on the Memory Tests analysed by the Mann-Whitney Non-Parametric test of Variance.

Test	Group	*n*	Mean Rank	Sum of Ranks	Mean	s.d.	Asymp. Sig. (2 Tailed)
Map Memory	1. Current Users	14	21.21	297.00	18.714	3.688	.004*
	2. Recovering Addicts	17	11.71	199.00	14.000	4.373	-
	Total:	31	-	-	-	-	-
Picture- Number Test	1. Current Users	14	17.57	246.00	14.929	9.376	.380
	2. Recovering Addicts	17	14.71	250.00	12.294	7.465	-
	Total:	31	-	-	-	-	-
Auditory Number Span Test	1. Current Users	14	20.25	283.50	14.000	2.689	.018
	2. Recovering Addicts	17	12.50	212.50	10.824	3.779	-
	Total:	31	-	-	-	-	-

(Bonferroni Correction: threshold value adjusted to $p = .0167$)
s.d.,Standard deviation; *n*, number of participants.
*, indicates $p < 0.0167$

TABLE 3: Comparisons of Group 1 (Current Users) and Group 3 (Control Group) regarding the neurocognitive measures of Memory analysed using the Mann-Whitney Non-Parametric test of Variance.

Test	Group	*n*	Mean Rank	Sum of Ranks	Mean	s.d.	Asymp. Sig. (2 Tailed)
Map Memory	1. Current Users	14	15.39	215.50	18.714	3.688	.553
	3. Control Group	18	17.36	312.50	19.556	3.240	-
	Total:	32	-	-	17.388	4.485	-
Picture Number Memory	1. Current Users	14	11.57	162.00	14.929	9.376	.009*
	3. Control Group	18	20.33	366.00	23.444	8.082	-
	Total:	32	-	-	17.143	9.496	-
Auditory Number Span Test	1. Current Users	14	17.39	243.50	14.000	2.689	.633
	3. Control Group	18	15.81	284.50	13.944	3.472	-
	Total:	32	-	-	12.878	3.644	-

(Bonferroni Correction: Threshold Value Adjusted To $p = .0167$)
s.d.,Standard deviation; *n*, number of participants.
*, indicates $p < 0.0167$

TABLE 4: Comparisons of Group 2 (Recovering Addicts) and Group 3 (Control Group) regarding their scores on the neurocognitive measures of Memory analysed using the Mann-Whitney Non-Parametric test of Variance.

Test	Group	*n*	Mean Rank	Mean	s.d.	Asymp. Sig. (2 Tailed)
Map Memory Test	2. Recovering Addicts	17	11.82	14.000	4.373	.001*
	3. Control Group	18	23.83	19.556	3.240	-
	Total:	35	-	-	-	-
		-	-	-	-	-
Picture Number Test	2. Recovering Addicts	17	11.85	12.294	7.465	.001*
	3. Control Group	18	23.81	23.444	8.082	-
	Total:	35	-	-	-	-
Auditory Number Span	2. Recovering Addicts	17	13.88	10.824	3.779	.020
	3. Control Group	18	21.89	13.944	3.472	-
	Total:	35	-	-	-	-

(Bonferroni Correction: threshold value adjusted to $p = .0167$)
s.d.,Standard deviation; *n*, number of participants.
*, indicates $p < 0.0167$

Data analysis

Demographic information was used entirely for the purposes of inclusion and exclusion in the study and not analysed. Raw scores obtained from the neuropsychological test battery were statistically analysed using non-parametric tests of variance due to a non-assumption of normality resulting from small sample sizes in the present research.

Raw scores were obtained from the paper and pencil tests administered and these scores were initially subjected to multivariate data analysis using the non-parametric Kruskal-Wallis test in which a significance level of $p = .05$ was applied. If the Kruskal-Wallis Test indicated significant results, further statistical analysis was utilised to determine which particular measures were most sensitive to methamphetamine dependence amongst the three groups. This analysis was performed using the non-parametric Mann-Whitney *U*-test with a Bonferroni correction adjusting the *P* - value from $p = .05$ to $p = .0167$ for all the results. This was done to safeguard against multiple tests of statistical significance on the same data falsely giving the appearance of significance.

Ethical considerations

The research was conducted in accordance with the approved research protocol of the University of Johannesburg. Informed consent was obtained from participants before they took part in the testing, and participation was voluntary and fully confidential. Participants were also advised of the purpose, expected duration and procedures involved in the research, and their right to withdraw from the research at any time, in accordance with the Code of Research Ethics of the Professional Board for Psychology and the Human Sciences Research Council.

Participants were offered debriefing following the study, and the opportunity to obtain the results of the research on completion of the study will be provided. This study is non-intrusive, non-deceptive and does not endanger the participants physically or emotionally. In order to ensure that any incentives would not interfere with the treatment and development of the participants, cash incentives were excluded.

Results

All three neuropsychological tests of Memory displayed significance on the Kruskal-Wallis non-parametric test of variance and as such were further analysed using the Mann-Whitney *U*-test and the results are displayed below.

Table 2 results illustrate that the only statistically significant difference in the population mean ranks lies in the Map Memory Test of the Kit of Factor-Referenced Cognitive Tests (KFRCT) (p =.004) (Average Group 1 = 18.714 versus Group 2 = 14.000)). Group 1, the current users of methamphetamine, therefore significantly outperforms Group 2, the recovering methamphetamine addicts, on the visual recall and recognition aspects of the Map Memory test from the KFRCT.

Table 3 illustrates that Group 3 (Control Group) performed better than Group 1 (Current Users) in only one test of memory – visual acquisition and retention Picture Number Test of the KFRCT (.009) (Average Group 1 = 14.929 versus Group 3 = 23.444).

Table 4 illustrates that Group 3 (Control Group) significantly outperformed Group 2 (Recovering Addicts) on the visual acquisition and retention test of Map Memory [(p =.001) (Average Group 2 = 14.000 versus Group 3 =19.556)] and the recall and recognition Picture-Number Test [(p =.001) (Average Group 2 =12.294 versus Group 3 = 23.444)] from the KFRCT.

It is noteworthy that no groups displayed any significant differences on the Auditory Number Span test from the KFRCT.

Discussion

The neurocognitive subtests that assessed memory and displayed significant results in this research are the map memory and picture-number association. *Map Memory* assesses visual recall and recognition (Ekstrom *et al*. 1987). The *Picture-Number Association test* assesses visual learning (acquisition) and memory (retention). No significant results were found on the short-term auditory memory storage and retrieval functions assessed by the *Auditory Number Span test* (Ekstrom *et al*. 1987).

The overall current findings of this research therefore indicate that those individuals currently using methamphetamine and the control group outperformed the abstinent recovering methamphetamine users in the short-term visual-recognition and recall assessment of memory. The drug naïve control subjects also performed significantly better than both the current and recovering methamphetamine users on tests of visual learning (acquisition) and retention (memory). Based on these results the research posits that the efficacy of the control group's storage and retrieval of information from intermediate visual memory can be considered significantly better than both the current and recovering users of methamphetamine.

Corroboration of the findings of this research, that recovering methamphetamine addicts are impaired in short-term visual recognition and recall, and visual acquisition and retention is found in Moon *et al*. (2007:6) who found selective damage on visual memory in abstinent methamphetamine users. Kalechstein *et al*. (2003:217) also found that the abstinent methamphetamine users performed poorly relative to the control group on non-verbal measures of memory and learning, although not significantly so.

This research posits that the memory deficits experienced by both the current and recovering users of methamphetamine are due to their methamphetamine exposure and its resultant deleterious effects in user's brain morphology and chemistry.

Structural and functional changes in the brain areas associated with memory due to methamphetamine neurotoxicity are believed to underlie the memory deficits experienced by methamphetamine users. The loss of cognitive functionality in these areas of memory is mirrored in literature and is believed to be attributed, in part, to the considerable loss of neurotransmitter functionality in the brain. Projections from the substantia nigra to the basal ganglia are part of the dopaminergic pathway, and dopamine appears to be essential to the functioning of the basal ganglia and therefore may have an indirect role in memory formation (Kolb & Whishaw 2003:468, 480). Therefore a loss of dopamine production and stimulation, which occurs due to methamphetamine cessation, may result in deleterious effects in memory formation and, according to the results of this study's, visual and visual associative short-term recognition and recall. The current users of methamphetamine, however, are still experiencing the juxtaposition of the stimulating and supplemental effect of methamphetamine. These users are therefore seen to be not as severely affected in terms of the cognitive function of memory whilst they are still using the drug as it promotes dopamine production and stimulation, which helps stave off the negative cognitive effects of the drug. However, prolonged chronic use of methamphetamine leads to greater neurotoxicity and once sober from the drug these users are likely to experience greater impairments than those who stopped using methamphetamine earlier.

The current users of methamphetamine, however, were significantly outperformed in the short-term explicit associative visual memory test, the Picture-Number Test, by the control subjects. As this test assesses associative memory and the storage and efficacy of retrieval of information from intermediate visual memory (Ekstrom *et al*. 1987:93) it can be said that the current users of methamphetamine suffer impairment in these areas of memory. This impairment, as noted in comparison to a drug-naïve population, can therefore probably be considered as a direct result of the current use of methamphetamine in this experimental population.

Literature supports this assertion as this type of impairment has been seen in another study with methamphetamine users in which the cognitive deficit was apparent in the more

difficult memory tasks such as recall tasks – that rely heavily on retrieval information – than in recognition tasks (Simon *et al*. 2000:229). The study by Simon *et al*. (2000:222) is one of few that deals with current users of methamphetamine in comparison to non-drug using control subjects. The authors found a pattern of memory functioning consistent with a mild generalised retrieval deficit often found in older adults suggesting that methamphetamine has a similar degenerative effect on the brain as aging does. The research conducted by Simon *et al*. (2000:227) also supports the overall findings of this study as both the current and recovering methamphetamine users experienced significant difficulty in comparison to the control subjects on the more difficult memory task of associative visual memory.

Contradictory literature by Kalechstein *et al*. (2003:217) exists to refute the findings of the current study. They found that the methamphetamine users performed significantly poorer than the control group on measures of verbal learning and memory. However, the authors concede that their participants may have still been experiencing withdrawal symptoms, which may have impaired the recovering methamphetamine user's auditory memory, and thus confounded the study results.

McKetin and Mattick's (1998:181) research both supports and refutes the findings of the present research. They found that high dependence illicit amphetamine users were impaired on measures of verbal memory in comparison to the control subjects, whilst the low dependence group displayed no impairment. The implications of these findings are that memory impairment in methamphetamine users is related to the reported severity of drug use. The current research made no provision for controlling the amount of methamphetamine use and could not label its users as high or low dependence on the drug and therefore cannot claim to compare its results fairly against those of McKetin and Mattick (1998:181).

It has been discussed that neither the current methamphetamine users – recovering methamphetamine users – nor the control group had significant deficits in short-term auditory memory in comparison to each other. Therefore the storage and retrieval of information in short-term auditory memory is not significantly affected by methamphetamine use. The lack of differences in short-term auditory memory storage and retrieval between the current users of methamphetamine, recovering users and the control subjects has been both supported by Moon *et al*. (2007). The authors found that methamphetamine causes selective damage on visual, but not verbal memory in methamphetamine users because visual memory tasks are more sensitive to executive dysfunction (Moon *et al*. 2007:5). Therefore, the impairment may not be due to methamphetamine-related damage to the areas of the brain associated with visual memory (Moon *et al*. 2007:6). Verbal memory tasks are not as sensitive to executive dysfunction and therefore appear to remain unimpaired, even after methamphetamine exposure.

This is an important aspect of the existing and current research that has clinical implications. If recovering methamphetamine addicts enter rehabilitation with impaired visual memory but unimpaired auditory memory, rehabilitation programmes should perhaps be constructed around the current strengths of the recovering addicts, as opposed to their current weaknesses, and the programme should be more verbally detailed and less visually orientated.

Limitations of the study

It is necessary to interpret all findings in this research within identified limits. Complicating all studies on methamphetamine is the fact that most drugs rarely are used on their own but rather in conjunction with other substances (Gonzalez *et al*. 2007:188). Most methamphetamine addicts are poly-drug users (Barr *et al*. 2006:306) which results in the difficulty to distinguish whether the observed neurocognitive effects are due to methamphetamine specifically or to its interaction with additional drugs.

The length of abstinence in the recovering methamphetamine addicts is another limitation as this study made use of recovering addicts with a minimum of one month of sobriety. The soundness of this research could be improved by using longer term abstinent methamphetamine users in order to fully determine the extent of the impairment in a longitudinal study design. Additionally, the group sizes in this research limit the ability to generalise the findings. The small group sizes are limited due to the resources required to attain the data, and the sensitive nature of the topic researched. Many other studies on methamphetamine and cognition (Johanson *et al*. 2006:327–338; Kalechstein *et al*. 2003:218; McKetin & Mattick 1998:181–184; Moon *et al*. 2007:1–9) suffer from the same limitation, indicating the complex and difficult nature of the research topic.

Finally, although this research did canvass three separate provinces, it was limited by both time and resources to these provinces and is thus not a fully representative study of the South African population.

Recommendations

In view of the results of this research, the study recommends that it would be a valuable undertaking to research the full extent of methamphetamine use in South Africa, in order to establish any province-particular patterns of use or country-specific consequences or recommendations. The relationship between the Western Cape and its high methamphetamine dependence rates, with particular focus placed on the greatest number of methamphetamine users in this province should be of primary concern.

The recognition of the link between methamphetamine use and its link to Human Immunodeficiency Virus (HIV) infection in South Africa should also be explored further. Recognition and knowledge of the risk behaviours of heterosexual and homosexual methamphetamine users

should stimulate prevention efforts that will ultimately help to slow the spread of HIV in the heterosexual population (Semple, Patterson & Grant 2004:810). Indeed, it is believed that effective treatment for methamphetamine addiction and dependence may be one of the most important strategies in reducing the spread of HIV and other, associated communicable diseases (Shoptaw *et al*. 2002).

Finally, to date there are few longitudinal studies concerning the long-term effects of methamphetamine in human beings with particular reference to potential recovery of cognitive functioning. Longitudinal descriptive studies are needed to follow methamphetamine users throughout the course of their use and recovery in order to ascertain the strategies they use to moderate or discontinue their drug abuse (Luna 2001:121). These studies will provide a comprehensive view of the methamphetamine patient and the clinical course of methamphetamine abuse and dependence. Additionally they will aid in determining the extent of cognitive, social, occupational and behavioural recovery and length of time required to achieve this recovery amongst long-term abstinent methamphetamine users. Suggested time periods for these studies are 3, 6, 12 and 24 months abstinence periods.

Conclusion

This research shows that methamphetamine negatively impacts an individual's memory functioning, both in current users and in rehabilitating users, to varying degrees. The cessation of methamphetamine use is met with a marked increase in cognitive impairment in comparison to the cognitive deficit noted in current methamphetamine users. Additionally, this degree of impairment is manifest in comparison to a matched control group.

This research also asserts that the memory functions of short-term visual recall and recognition, learning and retention are increasingly negatively impacted by methamphetamine use, more so than verbal or auditory memory. The impairment in visual learning, retention, recognition and recall as juxtaposed by the lack of deficit in short-term auditory memory has vast implications for the efficacy of traditional literature-based intervention and rehabilitation programmes. These would not be efficacious as the recently sober and recovering addicts would be impaired in their ability to memorise or even retain simple visual information. The majority of information presented in treatment programmes is in literature or coursework form that is provided to them visually. Therefore workshops and seminars, which include much verbal discussion, may be better suited to the rehabilitation situation.

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Competing interest

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Author's contributions

C.V.W. (University of Johannesburg) was the project leader, responsible for the study design and execution, and wrote the article; A.D.S. (University of Johannesburg) was responsible for supervision of the research project.

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EXHIBIT E

National Institute on Drug Abuse
The Science of Drug Abuse & Addiction

DrugFacts: Methamphetamine

Print

Revised March 2010

Methamphetamine is a central nervous system stimulant drug that is similar in structure to amphetamine. Due to its high potential for abuse, methamphetamine is classified as a Schedule II drug and is available only through a prescription that cannot be refilled. Although methamphetamine can be prescribed by a doctor, its medical uses are limited, and the doses that are prescribed are much lower than those typically abused. Most of the methamphetamine abused in this country comes from foreign or domestic superlabs, although it can also be made in small, illegal laboratories, where its production endangers the people in the labs, neighbors, and the environment.

How Is Methamphetamine Abused?

Methamphetamine is a white, odorless, bitter-tasting crystalline powder that easily dissolves in water or alcohol and is taken orally, intranasally (snorting the powder), by needle injection, or by smoking.

How Does Methamphetamine Affect the Brain?

Methamphetamine increases the release and blocks the reuptake of the brain chemical (or neurotransmitter) dopamine, leading to high levels of the chemical in the brain—a common mechanism of action for most drugs of abuse. Dopamine is involved in reward, motivation, the experience of pleasure, and motor function. Methamphetamine's ability to release dopamine rapidly in reward regions of the brain produces the intense euphoria, or "rush," that many users feel after snorting, smoking, or injecting the drug.

Chronic methamphetamine abuse significantly changes how the brain functions. Noninvasive human brain imaging studies have shown alterations in the activity of the dopamine system that are associated with reduced motor skills and impaired verbal learning.[1] Recent studies in chronic methamphetamine abusers have also revealed severe structural and functional changes in areas of the brain associated with emotion and memory,[2,3] which may account for many of the emotional and cognitive problems observed in chronic methamphetamine abusers.

Repeated methamphetamine abuse can also lead to addiction—a chronic, relapsing disease characterized by compulsive drug seeking and use, which is accompanied by chemical and molecular changes in the brain. Some of these changes persist long after methamphetamine abuse is stopped. Reversal of some of the changes, however, may be observed after sustained periods of abstinence (e.g., more than 1 year).[4]

What Other Adverse Effects Does Methamphetamine Have on Health?

Taking even small amounts of methamphetamine can result in many of the same physical effects as those of other stimulants, such as cocaine or amphetamines, including increased wakefulness, increased physical activity, decreased appetite, increased respiration, rapid heart rate, irregular heartbeat, increased blood pressure, and hyperthermia.

Long-term methamphetamine abuse has many negative health consequences, including extreme weight loss, severe dental problems ("meth mouth"), anxiety, confusion, insomnia, mood disturbances, and violent behavior. Chronic methamphetamine abusers can also display a number of psychotic features, including paranoia, visual and auditory hallucinations, and delusions (for example, the sensation of insects crawling under the skin).

Transmission of HIV and hepatitis B and C can be consequences of methamphetamine abuse. The intoxicating effects of methamphetamine, regardless of how it is taken, can also alter judgment and inhibition and can lead people to engage in unsafe behaviors, including risky sexual behavior. Among abusers who inject the drug, HIV/AIDS and other infectious diseases can be spread through contaminated needles, syringes, and other injection equipment that is used by more than one person. Methamphetamine abuse may also worsen the progression of HIV/AIDS and its consequences. Studies of methamphetamine abusers who are HIV-positive indicate that HIV causes greater neuronal injury and cognitive impairment for individuals in this group compared with HIV-positive people who do not use the drug.[5,6]

What Treatment Options Exist?

Currently, the most effective treatments for methamphetamine addiction are comprehensive cognitive-behavioral interventions. For example, the Matrix Model—a behavioral treatment approach that combines behavioral therapy, family education, individual counseling, 12-step support, drug testing, and encouragement for nondrug-related activities—has been shown to be effective in reducing methamphetamine abuse.[7] Contingency management interventions, which provide tangible incentives in exchange for engaging in treatment and maintaining abstinence, have also been shown to be effective.[8] There are no medications at this time approved to treat methamphetamine addiction; however, this is an active area of research for NIDA.

How Widespread Is Methamphetamine Abuse?

Monitoring the Future Survey

Methamphetamine use among teens appears to have dropped significantly in recent years, according to data revealed by the 2009 Monitoring the Future survey. The number of high-school seniors reporting past-year†† use is now only at 1.2 percent, which is the lowest since questions about methamphetamine were added to the survey in 1999; at that time, it was reported at 4.7 percent. Lifetime use among 8th-graders was reported at 1.6 percent in 2009, down significantly from 2.3 percent in 2008. In addition, the proportion of 10th-graders reporting that crystal methamphetamine was easy to obtain has dropped to 14 percent, down from 19.5 percent 5 years ago.

Methamphetamine Prevalence of Abuse
Monitoring the Future Survey, 2009

	8th grade	10th grade	12th grade
Lifetime**	1.6%	2.8%	2.4%
Past Year	1.0%	1.6%	1.2%
Past Month	0.5%	0.6%	0.5%

National Survey on Drug Use and Health***

According to the 2008 National Survey on Drug Use and Health, the number of past-month methamphetamine users age 12 and older decreased by over half between 2006 and 2008. Current (past-month) users were numbered at 731,000 in 2006, 529,000 in 2007, and 314,000 in 2008. Significant declines from 2002 and 2008 also were noted for lifetime and past-year use in this age group.

From 2002 to 2008, past-month use of methamphetamine declined significantly among youths aged 12 to 17, from 0.3 percent to 0.1 percent, and young adults aged 18 to 25 also reported significant declines in past-month use, from 0.6 percent in 2002 to 0.2 percent in 2008.

Other Information Resources

For more information on the effects of methamphetamine abuse and addiction, visit www.drugabuse.gov/drugpages/methamphetamine.html.

To find publicly funded treatment facilities by State, visit www.findtreatment.samhsa.gov.

Notes

* These data are from the 2008 Monitoring the Future survey, funded by the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, and conducted by the University of Michigan's Institute for Social Research. The study has tracked 12th-graders' illicit drug abuse and related attitudes since 1975; in 1991, 8th- and 10th-graders were added to the study.

** "Lifetime" refers to use at least once during a respondent's lifetime. "Past year" refers to use at least once during the year preceding an individual's response to the survey. "Past month" refers to use at least once during the 30 days preceding an individual's response to the survey.

*** NSDUH (formerly known as the National Household Survey on Drug Abuse) is an annual survey of Americans age 12 and older conducted by the Substance Abuse and Mental Health Services Administration. Copies of the latest survey are available at www.samhsa.gov and from NIDA at 877–643–2644.

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EXHIBIT F

Association of Dopamine Transporter Reduction With Psychomotor Impairment in Methamphetamine Abusers

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Objective: Methamphetamine is a popular and highly addictive drug of abuse that has raised concerns because it has been shown in laboratory animals to be neurotoxic to dopamine terminals. The authors evaluated if similar changes occur in humans and assessed if they were functionally significant.

Method: Positron emission tomography scans following administration of [^{11}C]d-threo-methylphenidate (a dopamine transporter ligand) measured dopamine transporter levels (a marker of dopamine cell terminals) in the brains of 15 detoxified methamphetamine abusers and 18 comparison subjects. Neuropsychological tests were also performed to assess motor and cognitive function.

Results: Methamphetamine abusers showed significant dopamine transporter reduction in the striatum (mean differences of 27.8% in the caudate and 21.1% in the putamen) relative to the comparison subjects; this reduction was evident even in abusers who had been detoxified for at least 11 months. Dopamine transporter reduction was associated with motor slowing and memory impairment.

Conclusions: These results provide evidence that methamphetamine at dose levels taken by human abusers of the drug leads to dopamine transporter reduction that is associated with motor and cognitive impairment. These results emphasize the urgency of alerting clinicians and the public of the long-term changes that methamphetamine can induce in the human brain.

(Am J Psychiatry 2001; 158:377–382)

The rapidly escalating abuse of methamphetamine in the United States (1) imposes a sense of urgency for understanding its effects on the human brain and its medical consequences. Methamphetamine is a particularly problematic drug in that it is not only highly addictive (2) but also can be manufactured by small clandestine laboratories, making the control of methamphetamine supplies difficult (3). As a result, epidemic pockets of methamphetamine abuse have recently developed in different areas of California and in sections of the southern and midwestern United States (4). This rise in methamphetamine abuse has also been reported for other areas in the world (5). As methamphetamine abuse rises, concern about its potential neurotoxic effects to the human brain increases, since methamphetamine administration in laboratory animals has resulted in profound and long-lasting toxicity to the brain. Particular damage has been documented in dopamine terminals (6, 7). Since the doses of methamphetamine administered to laboratory animals differ from those used by human abusers of the drug, it has been unclear whether similar deficits occur in human methamphetamine abusers. To our knowledge, only two studies with human data have been published: a postmortem study of 12 methamphetamine abusers (8) and an imaging study of six methamphetamine abusers (9). These studies reported dopamine transporter reductions in the brain, which suggests that methamphetamine at the doses abused by humans also affects the dopamine terminals. However, there are no data on the functional significance of these changes. The purpose of this study was to assess if we could document the dopamine transporter changes and assess their functional significance in a larger group of detoxified methamphetamine abusers.

Methamphetamine abusers and comparison subjects without a history of drug abuse underwent positron emission tomography (PET) scans following administration of [^{11}C]*d-threo*-methylphenidate (a dopamine transporter ligand [10]) to measure dopamine transporters, which serve as a marker for dopamine cell terminals. We also administered a battery of neuropsychological tests to assess the effects of methamphetamine abuse on motor activity and cognition.

Method

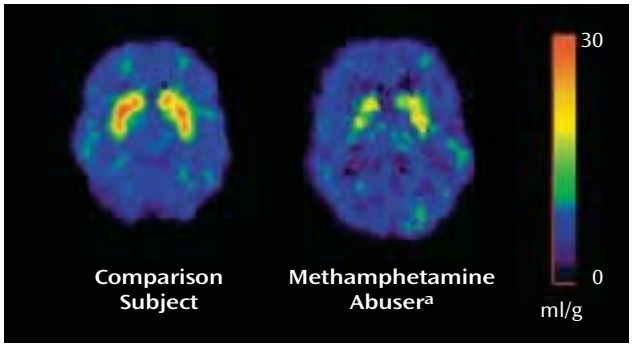
Subjects

Fifteen subjects (six men and nine women; mean age=32 years [SD=7], mean IQ=100 [SD=9]) who fulfilled DSM-IV criteria for

TABLE 1. Methamphetamine Use History of 15 Detoxified Abusers Administered [^{11}C]d-threo-Methylphenidate for Assessment of Dopamine Transporter Levels

Drug Use Variable	Methamphetamine Abusers (N=15)		
	Mean	SD	Range
Duration (years)	11	6	
Amount			
Average daily dose (g)	1.6	3.0	0.3–10.0
Cumulative (g)	12290	22396	514–86400
Time since last use (months)	5.9	9.0	0.5–36.0
	N	%	
Mode of intake			
Smoking and snorting	11	73	
Intravenous injection and snorting	2	13	
Smoking only	2	13	

FIGURE 1. Striatal Distribution Volume of the Dopamine Transporter Ligand [^{11}C]d-threo-Methylphenidate in a 33-Year-Old Male Comparison Subject and a 33-Year-Old Male Methamphetamine Abuser



[a] PET scan was performed 80 days after detoxification.

methamphetamine dependence were studied. Characteristics of the drug use history for these subjects are presented in Table 1. The subjects were included in the study if their average methamphetamine use involved at least 0.5 g/day, at least 5 days per week, for at least 2 years. Subjects were also required to have abstained from methamphetamine use for at least 2 weeks, which we confirmed by conducting a urine toxicology screening examination. Subjects were excluded from the study if they were seropositive for HIV or had a history of a comorbid psychiatric or neurological disorder, medical illness, current or past history of drug dependence (other than methamphetamine or nicotine), or a history of head trauma. Subjects were recruited from drug rehabilitation centers in the Los Angeles area. Detailed medical and drug use histories and results of physical, neurological, and psychiatric evaluations (conducted by L.C.) were independently corroborated at Brookhaven National Laboratory (by G.-J.W.). Blood test screenings, including HIV serology, were conducted to determine whether abnormalities were present. Twelve of the methamphetamine abusers had last used methamphetamine within 6 months of the study (range=2–24 weeks); the other three had not used it for at least 11 months (range=11–36).

Comparison subjects were 18 healthy volunteers (12 men and six women; mean age=31 years [SD=7], mean IQ=107 [SD=10]) who responded to a local advertisement. Exclusion criteria were the same as those for the methamphetamine abusers except the current or past history of drug problems included methamphetamine abuse. A complete medical and psychiatric examination was performed to ensure lack of medical, psychiatric, or neurological disease (G.-J.W., D.F.). The same screening laboratory tests as those given to the methamphetamine abusers (except for HIV serology) were used.

No subject was taking medications at the time of the study, and prescan urine tests were done to ensure the absence of psychoactive drug use in both the methamphetamine abusers and the comparison subjects.

Written informed consent was obtained from the subjects after the procedures had been fully explained. The study was approved by the institutional review boards at Brookhaven National Laboratory, the State University of New York at Stony Brook, and the Harbor-UCLA Medical Center.

Neuropsychological Evaluation

Within 2 weeks of the PET scans, we administered a neuropsychological battery to the methamphetamine abusers that was designed to include measures sensitive to functional deficits of the frontal lobe and the striatum (11). Five areas were assessed. First, motor function was rated by performance on two measures: the Timed Gait task, in which gross motor function is assessed by having the subject walk as fast as possible in a straight line, and the Grooved Pegboard task, in which fine motor coordination is assessed by having the subject insert pegs into small holes angled in different directions as quickly as possible. Second, attention was rated by performance on four tasks: the California Computerized Assessment Package (12), in which the subject responds as fast as possible to numbers and letters on a computer screen; the Symbol Digit Modalities Test, in which the subject matches numbers with symbols; the Trail Making Test, in which the subject draws lines connecting consecutive numbers or numbers that alternate with letters; and the Stroop Interference Test, in which the subject reads color names printed in incongruent ink colors and has to suppress the tendency to say the word instead of the color. Third, memory was rated by performance on the Rey Auditory Verbal Learning Test, in which the subject has to learn and recall lists of unrelated words immediately, after a time delay, and after a distractor. Fourth, depressive symptoms were measured with the Center for Epidemiologic Studies Depression Scale (13). Last, general intelligence was rated with the New Adult Reading Test Revised, which gives an estimate of verbal intelligence.

Scans

PET scans were performed by using a CTI 931 scanner (Siemens, Knoxville, Tenn.) (spatial resolution: 6 × 6 × 6.5-mm full width at half maximum). Dynamic scans that followed previously described procedures (11) were started after intravenous injection of 4–8 mCi of [^{11}C]*d-threo*-methylphenidate (specific activity >0.4 Ci/μmol at time of injection); the scans lasted a total of 84 minutes.

Image Analysis

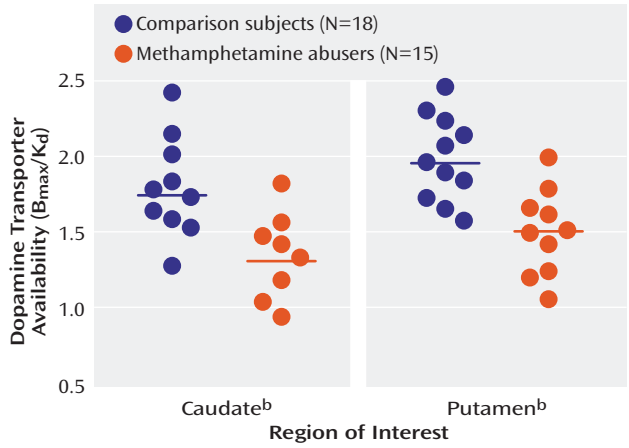
Regions in the striatum (caudate, putamen) were obtained from three sequential planes and in the cerebellum from two sequential planes and were drawn on the averaged emission scans (activity between 10 and 84 minutes). Regions in the striatum were selected in multiple planes to increase the reproducibility of the measures (14). The regions were then projected to the dynamic emission scans to obtain tissue time activity curves. By means of a graphical analysis technique for reversible systems (15), these tissue time activity curves along with the time activity curves for unchanged tracer in plasma were used to calculate in the striatum and cerebellum the transfer constant (K_1) from plasma to brain of [^{11}C]*d-threo*-methylphenidate and its distribution volume, which corresponds to the equilibrium measurement of the ratio of tissue concentration to plasma concentration. The ratio of distribution volume in the striatum to that in the cerebellum, which corresponds to (B_{max}/K_d) + 1 and is insensitive to

TABLE 2. Regional Dopamine Transporter Measures in Detoxified Methamphetamine Abusers and Comparison Subjects With No History of Drug Abuse

	Methamphetamine Abusers (N=15)						Comparison Subjects (N=18)					
	K_1[a]		Distribution Volume[b]		B_{max}/K_d[c]		K_1[a]		Distribution Volume[b]		B_{max}/K_d[c]	
Region	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Caudate	0.61	0.2	22.8[d]	3.7	1.3[d]	0.2	0.58	0.1	29.8	3.9	1.8	0.3
Putamen	0.71	0.2	24.9[d]	4.2	1.5[d]	0.2	0.64	0.1	31.7	4.9	1.9	0.2
Cerebellum	0.46	0.2	9.8	1.2			0.49	0.1	10.8	1.4		

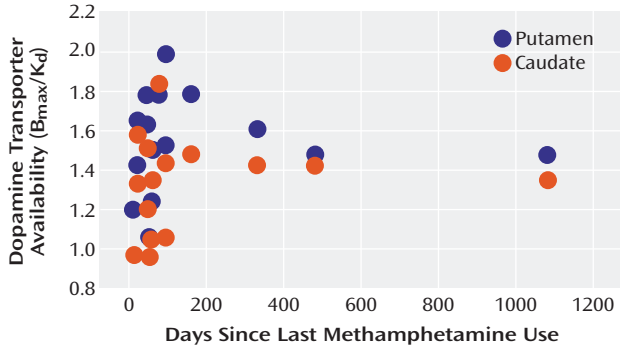
[a] Rate constant, a function of the transport of the radiotracer from plasma to tissue.
[b] Tissue/plasma concentration ratio for the dopamine transporter ligand [^{11}C]d-threo-methylphenidate.
[c] Estimate of dopamine transporter availability.
[d] Significantly different from the comparison subjects (two-tailed Student's t tests: t>4.7, df=31, p<0.0001).

FIGURE 2. Caudate and Putamen Dopamine Transporter Availability (B_{max}/K_d) in Detoxified Methamphetamine Abusers and Comparison Subjects With No History of Drug Abuse[a]



[a] Horizontal lines represent mean values. Some subjects had overlapping values.
[b] Significantly lower dopamine transporter availability in the methamphetamine abusers (t>4.7, df=31, p<0.0001).

FIGURE 3. Caudate and Putamen Dopamine Transporter Availability (B_{max}/K_d) in 15 Detoxified Methamphetamine Abusers by Days Since Last Methamphetamine Use



changes in cerebral blood flow (16), was used as model parameter of dopamine transporter availability.

Statistics

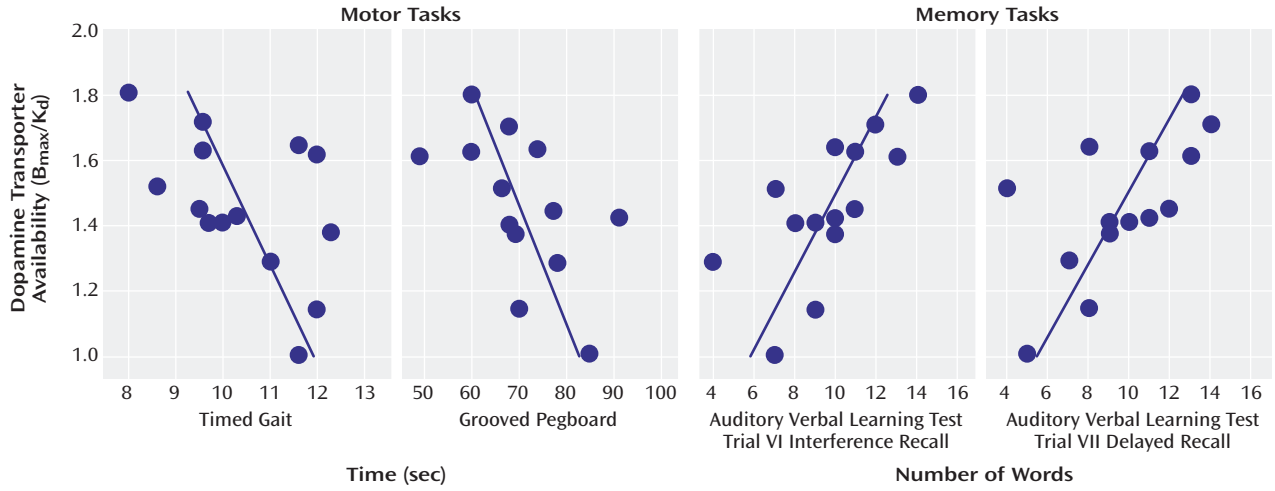
Differences between comparison subjects and methamphetamine abusers in K_1, distribution volume, and B_{max}/K_d were tested with unpaired Student's t tests (two-tailed). For the methamphetamine abusers, Pearson product-moment correlation analyses were performed between dopamine transporter measures and neuropsychological test scores, the years and doses of methamphetamine used, and days since last methamphetamine use. We hypothesized a priori an association between dopamine transporter levels and performance on the two motor and the three auditory verbal learning tasks, since abnormal results on these tests had been found in patients with Parkinson's disease in proportion to dopamine damage. A significance threshold was set at p<0.05. For exploratory analyses of the correlation between dopamine transporter levels and performance on the other seven neuropsychological tests, we set the significance threshold at p<0.007.

Results

The K_1 measures did not differ between the comparison subjects and the methamphetamine abusers, whereas the distribution volumes in the caudate and putamen, but not in the cerebellum, were significantly lower in the abusers (Figure 1, Table 2). Dopamine transporter availability (B_{max}/K_d) was significantly lower in the methamphetamine abusers than in the comparison subjects both in the caudate (27.8% difference) and in the putamen (21.1% difference) (Table 2). Figure 2 shows the dopamine transporter availability (B_{max}/K_d) values for the individual comparison subjects and methamphetamine abusers. A significant correlation between dopamine transporter level and years of methamphetamine use was found in the caudate (r=0.54, df=14, p<0.05). This correlation approached significance in the putamen (r=0.47, df=14, p<0.08), but no correlation was seen in these areas between dopamine transporter levels and methamphetamine dose (r<0.37, df=14, p>0.17). Dopamine transporter levels also were not significantly correlated with days since last methamphetamine use (r<0.11, df=14, p>0.70) (Figure 3).

Because the estimates of dopamine transporters in the caudate were strongly correlated with those in the putamen (r=0.75, df=14, p<0.0001), we averaged these two measures into a striatal value. The correlations between striatal dopamine transporters and performance on the neuropsychological tests for which we hypothesized an association a priori were significant for the motor tasks (Timed Gait task: r=0.53, df=14, p<0.05; Grooved Pegboard task: r=0.57, df=14, p<0.05) and for the verbal memory task (Auditory Verbal Learning Test, interference recall: r=0.70,

FIGURE 4. Association Between Striatal Dopamine Transporter Availability (B_{max}/K_d) and Neuropsychological Performance in Detoxified Methamphetamine Abusers[a]



[a] Some subjects had overlapping values.

df=14, p<0.005; delayed recall: r=0.64, df=14, p<0.01; immediate recall: r=0.58, df=14, p<0.05) (Figure 4). For the exploratory analyses, only the correlation with the Trail Making Test approached significance (r=0.61, df=14, p<0.05). None of the other tests were significantly correlated with striatal dopamine transporter level.

Discussion

This study documented significant dopamine transporter reduction in detoxified methamphetamine abusers relative to non-drug-abusing comparison subjects that was associated with poor motor and memory performance. Dopamine transporter reduction was observed as lower [^{11}C]*d-threo*-methylphenidate distribution volume and lower striatal dopamine transporter availability in the methamphetamine abusers relative to the comparison subjects. The fact that there were no differences in K_1 for [^{11}C]*d-threo*-methylphenidate between comparison subjects and methamphetamine abusers indicates that the reductions in distribution volume were not due to changes in tracer delivery. Dopamine transporter reduction was seen even in the three methamphetamine abusers who had been detoxified for at least 11 months. These findings are consistent with previous reports in human (8, 9) and nonhuman primates (17, 18) that have documented dopamine transporter changes after methamphetamine administration. The findings from these studies have implications in the treatment of methamphetamine abusers, for they suggest that interventions that improve dopamine brain function would benefit these individuals by improving motor and cognitive function.

The dopamine transporter reductions seen in the methamphetamine abusers were smaller than those found in patients with Parkinson's disease, in whom dopamine transporter reductions are proportional to disease severity and range between 36% and 71% (19–21). However, it should be noted that in three methamphetamine abusers, the dopamine transporter level fell within the range seen in patients with low-severity Parkinson's disease. It is likely that these three relatively young subjects did not have extrapyramidal symptoms because they were still able to compensate. Nonetheless, dopamine transporter reductions resulted in impaired motor performance on the Timed Gait and Grooved Pegboard tasks: the lower the dopamine transporter level, the slower the motor responses. A similar association, albeit for more severe pathology, has been reported for patients with Parkinson's disease studied with PET and [^{18}F]fluoro-L-DOPA (22). Thus, while the dopamine transporter reductions in the methamphetamine abusers may not have been severe enough to induce parkinsonian symptoms, our findings suggest that they resulted in impairment of motor function. Since significant dopamine transporter reductions occur both with age (6%–7% per decade) (23) and with methamphetamine abuse, it is possible that an interaction effect of methamphetamine abuse and aging may yield a higher risk for the development of parkinsonian symptoms in these abusers later in life.

The dopamine transporter reductions in the methamphetamine abusers also differed from those seen in patients with Parkinson's disease in that the magnitude of the reduction was similar in the caudate and putamen, whereas in Parkinson's disease, the putamen is more frequently affected than the caudate (24–26). This suggests different mechanisms for dopamine transporter reduction in methamphetamine abusers than in Parkinson's disease. Also, since the caudate is more involved than the putamen in cognitive operations (27), it is to be expected that in methamphetamine abusers dopamine transporter reduction will result in cognitive as well as motor impairment. In fact, in laboratory animals methamphetamine

induces motor (28) and learning and memory impairments (29). Moreover, the degree of dopamine transporter reduction in the methamphetamine abusers predicted both the motor as well as the memory changes; the lower the dopamine transporter levels, the worse their performance. Performance on the task used to assess verbal memory in this study (Auditory Verbal Learning Test) has also been found to be impaired in Parkinson's disease patients (30), whose performance, as was seen with the methamphetamine abusers, was found to be associated with dopamine transporter levels (31).

The dopamine transporter reductions in the methamphetamine abusers were smaller than those reported in animal studies, which have exceeded 50% (6, 7). This could be due to differences in doses and patterns of use, interspecies differences, or coadministration of other drugs. Of particular relevance may be the fact that most methamphetamine abusers are cigarette smokers, since nicotine has been shown to be protective against methamphetamine neurotoxicity (32). Thus, it is possible that one of the reasons why less dopamine transporter reduction was seen in the human methamphetamine abusers than has been reported in animal studies is that cigarette smoking may have provided some protection against methamphetamine-induced dopamine transporter reduction.

Studies in nonhuman primates have reported some recovery of dopamine terminal damage induced by methamphetamine abuse (18). Although our study was not designed to assess recovery, the fact that the length of methamphetamine detoxification was not correlated with dopamine transporter levels suggests that in human methamphetamine abusers, dopamine transporter losses do not recover significantly following 1 year of detoxification.

The dopamine transporter reductions in the methamphetamine abusers could reflect either a decrease in dopamine transporter expression or degeneration of dopamine terminals. While there is evidence from preclinical studies that methamphetamine induces dopamine terminal degeneration (33), a human postmortem study showed dopamine transporter reductions but not vesicular monoamine transporter reductions (9). Since vesicular monoamine transporters are more stable markers of dopamine terminals than dopamine transporters, this was interpreted as reflecting persistence of the dopamine terminal (8). Thus, further studies are required to determine if dopamine transporter reductions in humans are due to dopamine terminal degeneration. This study focused on the effects of methamphetamine on dopamine transporter levels, but animal studies have shown that methamphetamine also damages other neuronal types (6, 7). Thus, further studies are required to assess if methamphetamine disrupts neuronal systems other than dopamine in human subjects.

In summary, the results from this study provide evidence that methamphetamine at doses abused by humans leads to dopamine transporter reductions in the brain and that this reduction is associated with functional impairment. The fact that the dopamine transporter levels were lower even in subjects detoxified for at least 11 months suggests that methamphetamine's effects in the human brain may be long lasting. At present we do not know whether the dopamine transporter reductions reflect dopamine terminal damage or down-regulation of dopamine transporter expression; we also do not know whether this reduction may eventually resolve or whether it may increase vulnerability to Parkinson's disease or other neurodegenerative diseases. Thus, there is an urgent need to alert methamphetamine users to the consequences of their abuse and to develop treatments for these patients. Similarly, preventive measures are needed urgently to warn and educate the public of the damaging effects of methamphetamine to the human brain.

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EXHIBIT G

Cell

Acute Cannabinoids Impair Working Memory through Astroglial CB_1 Receptor Modulation of Hippocampal LTD

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SUMMARY

Impairment of working memory is one of the most important deleterious effects of marijuana intoxication in humans, but its underlying mechanisms are presently unknown. Here, we demonstrate that the impairment of spatial working memory (SWM) and in vivo long-term depression (LTD) of synaptic strength at hippocampal CA3-CA1 synapses, induced by an acute exposure of exogenous cannabinoids, is fully abolished in conditional mutant mice lacking type-1 cannabinoid receptors (CB_1R) in brain astroglial cells but is conserved in mice lacking CB_1R in glutamatergic or GABAergic neurons. Blockade of neuronal glutamate *N*-methyl-D-aspartate receptors (NMDAR) and of synaptic trafficking of glutamate α-amino-3-hydroxy-5-methyl-isoxazole propionic acid receptors (AMPAR) also abolishes cannabinoid effects on SWM and LTD induction and expression. We conclude that the impairment of working memory by marijuana and cannabinoids is due to the activation of astroglial CB_1R and is associated with astroglia-dependent hippocampal LTD in vivo.

INTRODUCTION

The treatments of pain, nausea, seizures, ischemia, cerebral trauma and tumors in humans and/or animals are some of the potential therapeutic applications of derivatives of the plant *Cannabis sativa* (marijuana) or synthetic cannabinoids (Lemberger, 1980; Robson, 2001; Brooks, 2002; Carlini, 2004; Hall et al., 2005). However, the potential therapeutic use of cannabis is limited by important side-effects associated with its use (Pacher et al., 2006). One of the major side effects of marijuana intoxication is the impairment of working memory in humans (Ranganathan and D'Souza, 2006) and animals (Lichtman and Martin, 1996; Hampson and Deadwyler, 2000; Nava et al., 2001; Varvel and Lichtman, 2002; Fadda et al., 2004; Hill et al., 2004; Wise et al., 2009), but the cellular mechanisms of this effect are presently not known.

Working memory is the ability to transiently hold and process information for reasoning, comprehension and learning, such as active thinking. Baddeley introduced a multicomponent model of human working memory with a central executive system responsible for information integration and coordination of two subsystems (Baddeley, 2003). One subsystem, the phonological loop, stores the sound of language while the other subsystem, the visuo-spatial sketch pad, stores visual (e.g., color) and spatial information (i.e., location). This theory suggests a key role of spatial processing in working memory

performance. Spatial working memory (SWM) in humans and animals requires online processing of information within many brain regions including the hippocampus (Hassabis et al., 2007; Kesner, 2007). The hippocampal excitatory CA3-CA1 synapses, which connect glutamatergic axons of CA3 pyramidal neurons, including the ipsilateral Schaffer collaterals and contralateral commissural fibers, with dendrites of CA1 pyramidal neurons (Witter and Amaral, 2004), have been proposed to play a key role in SWM (Rolls and Kesner, 2006).

Multiple forms of memory are likely subserved by activity- or experience-dependent long-term potentiation (LTP) and depression (LTD) of synaptic strength (Malenka and Bear, 2004). Chronic exposure of rats to cannabinoids impairs both LTP induction at CA3-CA1 synapses and hippocampal-dependent SWM (Hill et al., 2004), suggesting a link between LTP impairment and SWM impairment. This idea is supported by recent data that knockout of the AMPAR GluR1 subunit impairs both LTP induction at CA3-CA1 synapses and SWM (Sanderson et al., 2008). If LTP at CA3-CA1 synapses indeed contributes to SWM, LTD at these synapses may play a role in SWM impairment, because LTD could counteract LTP at the same synapses (Han et al., 2011).

Cannabinoid type-1 receptor (CB_1R), one of the most abundant G protein-coupled receptors in the brain (Herkenham et al., 1990), is found in both GABAergic and glutamatergic neurons in the hippocampal CA1 region (Herkenham et al., 1990; Kawamura et al., 2006; Marsicano and Lutz, 2006). Its main neuronal action is to inhibit presynaptic neurotransmitter release (Kano et al., 2009; Marsicano and Lutz, 2006). Indeed, cannabinoids can depress excitatory transmission at CA3-CA1 synapses in brain slices via activation of CB_1R (Misner and Sullivan, 1999; Hajos et al., 2001; Kawamura et al., 2006; Marsicano and Lutz, 2006; Takahashi and Castillo, 2006; Bajo et al., 2009; Serpa et al., 2009; Hoffman et al., 2010). Thus, cannabinoid-induced decrease of excitatory transmission might be related to SWM impairment. It is entirely unknown, however, whether cannabinoids are able to induce LTD at CA3-CA1 synapses in living animals and whether such in vivo LTD might contribute to SWM impairment induced by exogenous cannabinoids. In addition to the presence in neurons, CB_1R is also found in hippocampal astroglial cells and its activation, by stimulating Ca^{2+}-dependent release of glutamate, potentiates synaptic transmission at CA3-CA1 synapses in brain slices (Navarrete and Araque, 2010). However, the roles of astroglial CB_1R in the modulation of behavior and synaptic plasticity in living animals are not known.

In this study, we employed conditional mutagenesis, in vivo electrophysiology and behavioral tests to study the mechanism underlying the effect of cannabinoids on hippocampal-dependent SWM. Surprisingly, we found that activation of astroglial CB_1R, but not neuronal CB_1R, by exogenous cannabinoids mediates SWM impairment and LTD induction at CA3-CA1 synapses in vivo. Our data reveal an unanticipated hippocampal pathway linking astroglial activity, synaptic plasticity and memory processing, and define the specific mechanisms likely underlying cannabinoid-induced impairment of SWM in living animals.

RESULTS

Cannabinoids Induce In Vivo LTD at CA3-CA1 Synapses

In vivo recordings of field excitatory postsynaptic potentials (fEPSP) from CA3-CA1 synapses in anesthetized rats revealed that an i.p. injection of HU210 (0.05 or 0.1 mg/kg), a potent synthetic cannabinoid, or Δ^9-tetrahydrocannabinol (THC, 5 mg/kg), the major psychoactive ingredient of marijuana, decreased fEPSP amplitude to approximately 40% of the baseline levels (Figures 1A and 1G). Similar results were obtained after an intra-CA1 infusion of HU210 (Figures S1A and S1C). In studies hereafter, animals received an i.p. injection of 0.05 mg/kg of HU210 or 5 mg/kg of THC if not otherwise stated.

Cannabinoid-induced depression of synaptic transmission at CA3-CA1 synapses in brain slices is not defined as LTD, because it is fully reversed by application of CB_1R antagonists 10 min after cannabinoid application (Chevaleyre et al., 2006; Hajos et al., 2001; Kawamura et al., 2006). This indicates the requirement of a continuous activation of CB_1R for cannabinoid depression of transmission at CA3-CA1 synapses, a characteristic of transient synaptic depression but not of LTD (Chevaleyre et al., 2006). However, we observed that the decreased EPSP amplitude was blocked by injection of the selective CB_1R antagonist AM281 (3 mg/kg, i.p.) (Cui et al., 2001) 10 min before, but not 10 min after HU210 administration (Figures 1B and 1G), thus indicating LTD induction by cannabinoid exposure in vivo (hereafter referred to as CB-LTD). This idea is further supported by two lines of evidence. First, while synaptic transmission depression can be transient (in min) or long-lasting (i.e., LTD lasting > 24 h), a HU210 injection (0.1 mg/kg, i.p.) induced CB-LTD at CA3-CA1 synapses for > 24 hr in freely moving rats (Figures 1E and 1G), at a time where the acute effects of the drug should be decreased. Second, while the maintenance of late-phase LTD, but not early-phase LTD or transient synaptic transmission depression, requires new protein synthesis (Kelleher et al., 2004), administration of inhibitors of protein translation (anisomycin, 18 mg/kg, i.p.) (Puighermanal et al., 2009) or RNA transcription (actinomycin-D, 72 μg/12 μl, i.c.v.) (Manahan-Vaughan et al., 2000) 2 hr before HU210 injection selectively reversed the late-phase expression of CB-LTD (Figures 1C and 1G).

To identify if CB_1R expressed in the CA1 area contributes to CB-LTD at CA3-CA1 synapses, we applied adenoviral vectors-containing shRNA against CB_1R into the CA1 region 4 days prior to HU210 injection. shRNA CB_1R specifically knocked down CA1 expression of CB_1R (Figure 1F) and suppressed CB-LTD at CA3-CA1 synapses (Figures 1D and 1G). Interestingly, the cannabinoid effect seems to be specific for the CA3-CA1 pathway, because systemic HU210 did not induce CB-LTD at synapses of the perforant path onto dentate gyrus neurons (Figures S1B and S1C). Thus, in vivo cannabinoid exposure induces an in vivo LTD at CA3-CA1 synapses.

Neuronal CB_1R Is Dispensable for CB-LTD at CA3-CA1 Synapses

Glutamatergic presynaptic membranes of CA3-CA1 synapses contain CB_1R (Kawamura et al., 2006). To test whether CB-LTD

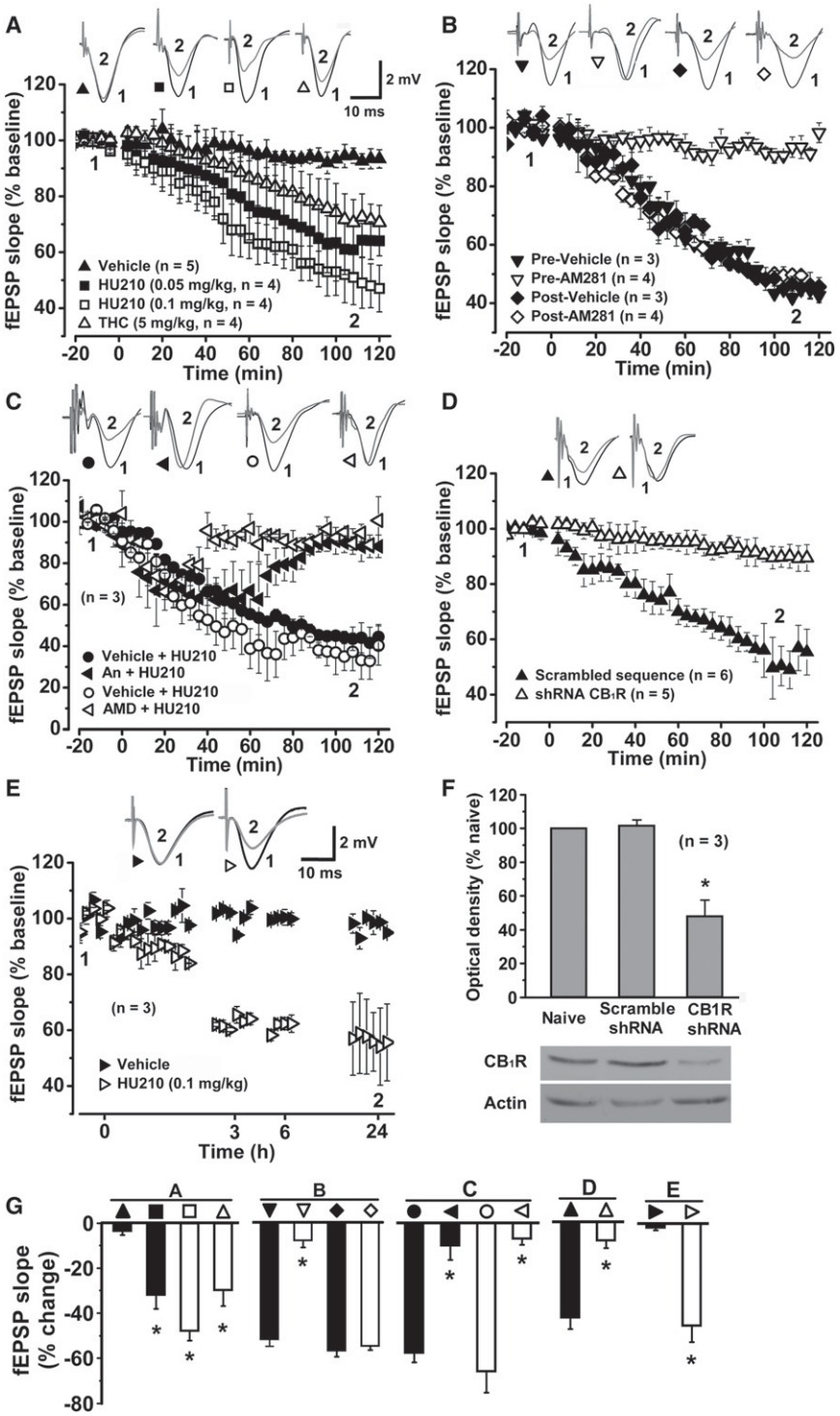

Figure 1. Cannabinoids Induce In Vivo LTD at CA3-CA1 Synapses

(A–E) Plots of normalized fEPSP slopes in anesthetized rats (A–D) or freely moving rats (E) show that cannabinoid injection at 0 min elicits CA1 LTD lasting for > 2 hr (A–D) or > 24 hr (E), which is blocked by AM281 administration 10 min before, but not 10 min after, HU210 injection (B), or by intra-CA1 infusion of shRNA CB_1R (D), and that anisomycin (An) and actinomycin-D (AMD) selectively reverse the late-phase expression of HU210-elicited LTD (C). Representative fEPSP traces before (1) and after (2) vehicle or cannabinoid injection are shown above each plot.

(F) Graph (top) and immunoblotting photos (bottom) show a reduction of CA1 CB_1R expression by shRNA CB_1R.

(G) Histogram summarizes the average percent change of fEPSP slope before (1) and after (2) vehicle or cannabinoid injection as depicted in panels (A)–(E).

All summary graphs show means ± standard error of the mean (SEM); n = numbers of animals recorded in each group (A–E) or numbers of experiments conducted (F) in each group. *p < 0.01 versus vehicle control, Bonferronni post-hoc test after one-way ANOVA (A: $F_{3,13} = 56.560$, $p < 0.01$; B: $F_{3,10} = 39.001$, $p < 0.01$; C: $F_{3,8} = 47.210$, $p < 0.01$; F: $F_{2,6} = 34.990$, $p < 0.01$) or t test.

See also Figure S1.

depends on "glutamatergic" CB_1R, we examined mutant mice carrying a selective deletion of the *CB_1R* gene in cortical and hippocampal glutamatergic principal neurons (Glu-*CB_1R*-KO) (Monory et al., 2006; Bellocchio et al., 2010). Surprisingly, THC induced a CB-LTD at CA3-CA1 synapses that was indistinguishable between wild-type mice and Glu-*CB_1R*-KO littermates (Figures 2A and 2C). We then determined the induction of CB-LTD in mutant mice carrying a selective deletion of the *CB_1R* gene in brain GABAergic neurons (GABA-*CB_1R*-KO), including CA1 GABAergic neurons (Monory et al., 2006; Bellocchio et al., 2010). Again, THC induced a CB-LTD at CA3-CA1 synapses that was indistinguishable between wild-type mice and GABA-*CB_1R*-KO littermates (Figures 2A and 2C). Thus, CB_1R expressed in glutamatergic or GABAergic neurons does not participate in this in vivo form of CB-LTD in the hippocampal CA1 region.

Astroglial CB_1R Mediates CB-LTD at CA3-CA1 Synapses

CB_1R is also functionally expressed in CA1 astrocytes (Navarrete and Araque, 2008). Therefore, astroglial CB_1R might play a role in CB-LTD at CA3-CA1 synapses. To directly address this issue, we generated tamoxifen-inducible conditional mutant mice specifically lacking CB_1R expression in astrocytes. "Floxed" *CB_1R* mutant mice (Marsicano et al., 2003) were crossed with transgenic mice expressing the inducible version of the Cre recombinase CreERT2 under the control of the promoter of the human glial fibrillary acidic protein (GFAP-CreERT2 mice,

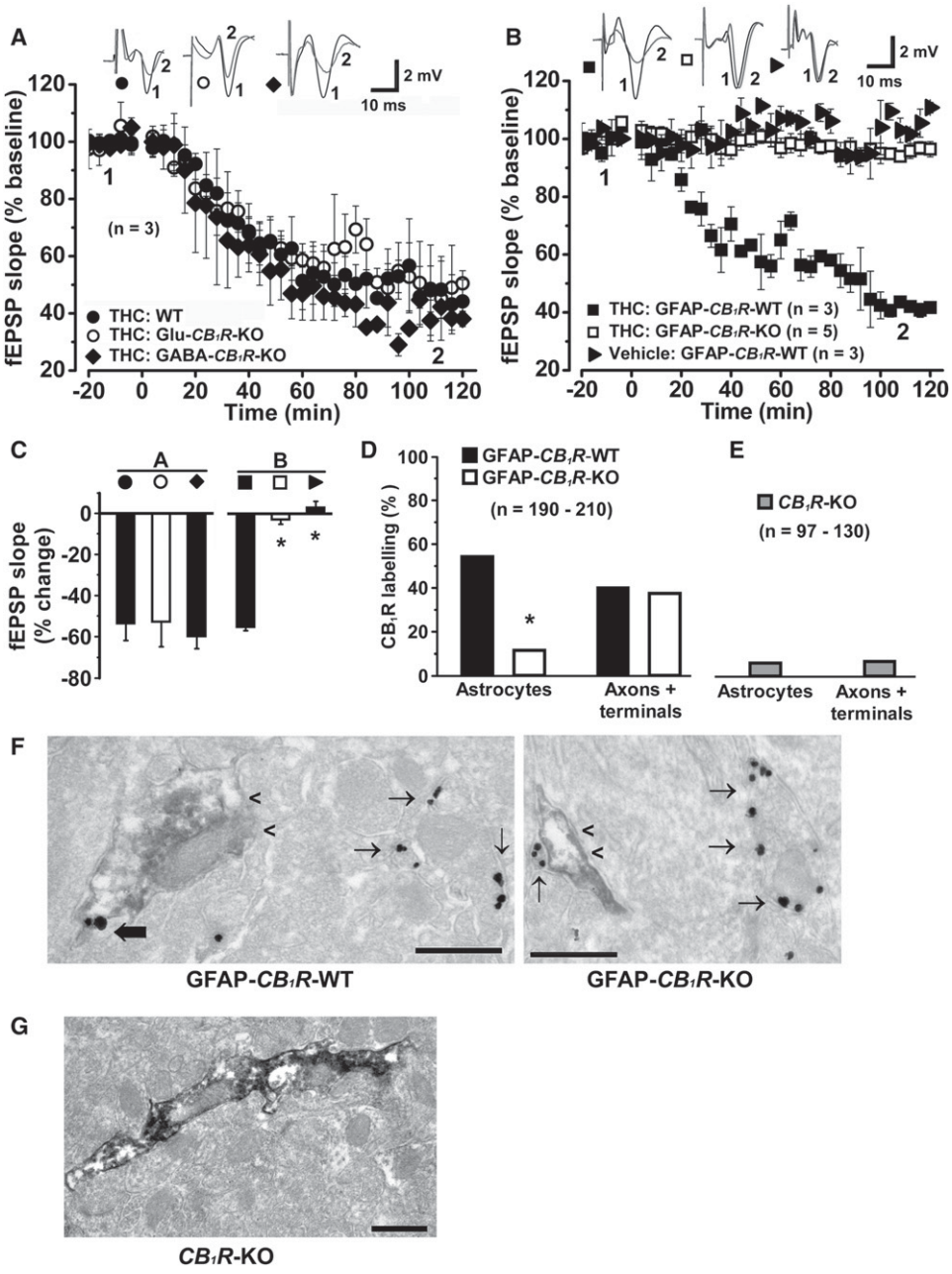

Figure 2. Cannabinoids Elicit CA1 LTD via Astroglial CB_1R but Not Neuronal CB_1R

(A and B) Plots of normalized fEPSP slopes in anesthetized mice show that THC injection at 0 min elicits CA1 LTD in wild-type (WT), Glu-*CB₁R*-KO and GABA-*CB₁R*-KO mice (A), but not in GFAP-*CB₁R*-KO mice (B). Representative fEPSP traces before (1) and after (2) treatment are shown above each plot.

(C) Histogram summarizes the average percent changes of fEPSP slope before (1) and after (2) treatment.

(D and E) Histograms summarize the percentage of CB_1R-labeled astrocytes and axons/terminals in GFAP-*CB₁R*-WT mice, GFAP-*CB₁R*-KO mice and *CB₁R*-KO mice.

(F) Electron microscopic images show a high density of CB_1R immunopositive silver grains (small arrows) in axons/terminals of both tamoxifen-treated GFAP-*CB₁R*-WT and GFAP-*CB₁R*-KO mice, and a low density of silver grains (large arrow) in DAB-stained astrocytes (arrowheads) of GFAP-*CB₁R*-WT mice but not of GFAP-*CB₁R*-KO littermates. The scale bar represents 500 nm.

(G) An electron microscopic image shows an absence of CB_1R immunopositive silver grains in astrocytes stained with peroxidase/DAB and axons. The scale bar represents 500 nm.

All summary graphs show means ± SEM; n = numbers of animals recorded (A, B) or numbers of positive immunoreactive profiles counted (D, E) in each group. *$p < 0.01$ versus control, Bonferroni post-hoc test after one-way ANOVA (A: $F_{2,6} = 68.603$, $p = 0.884$; B: $F_{2,8} = 42.009$, $p < 0.01$) or square Chi test (D).

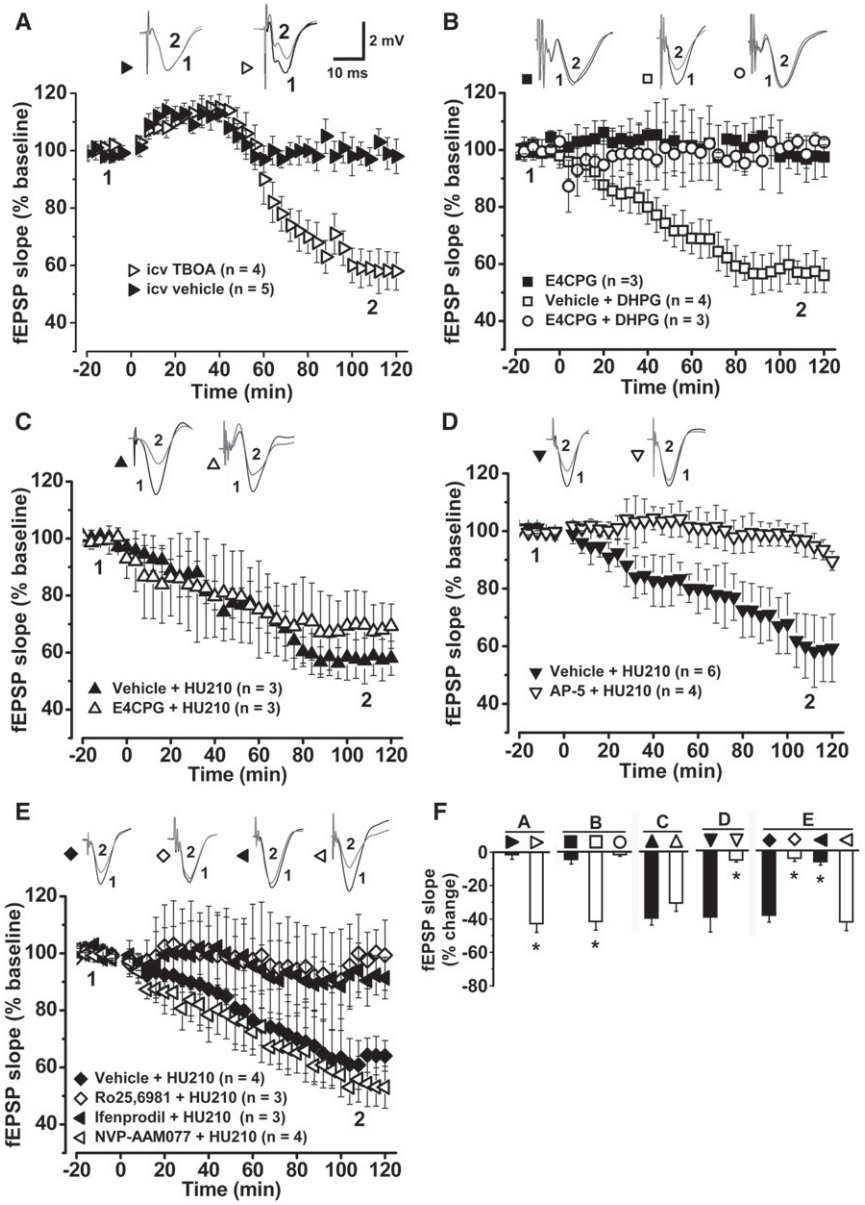

Figure 3. Cannabinoids Induce NMDAR-Dependent LTD at CA3-CA1 Synapses

(A–E) Plots of normalized fEPSP slopes in anesthetized rats are presented with representative fEPSP traces (above plots) before (1) and after (2) vehicle or drug injection. An i.c.v. injection of TBOA induces LTD (A). E4CPG, but not vehicle, blocks LTD induced by DHPG injection at 0 min (B) without significant effects on LTD induced by HU210 (C). Intra-CA1 application of AP-5 suppresses HU210-induced LTD (D). Systemic administration of Ro25-6981 and ifenprodil, but not NVP-AAM077, prevents HU210-induced LTD (E).

(F) Histogram summarizes the average percent change of fEPSP slope before (1) and after (2) drug or vehicle injection.

All summary graphs show means ± SEM; n = numbers of animals recorded in each group. *p < 0.01 versus control, Bonferronni post-hoc test after one-way ANOVA (B: $F_{2,7} = 36.090$, $p < 0.01$; E: $F_{3,10} = 40.409$, $p < 0.01$) or t test.

Hirrlinger et al., 2006) to eventually obtain the GFAP-*CB$_1$R*-KO mouse line. As compared to tamoxifen-treated wild-type littermate controls (GFAP-*CB$_1$R*-WT), GFAP-*CB$_1$R*-KO mice displayed a 79% reduction ($p < 0.01$) in the number of CA1 astrocytes labeled with a CB$_1$R antibody (Figures 2D and 2F), whereas only background levels were observed in constitutive *CB$_1$R*-KO mice (Figures 2E and 2G). Conversely, no difference ($p = 0.2293$) was observed between GFAP-*CB$_1$R*-WT and GFAP-*CB$_1$R*-KO mice in the number of CB$_1$R-labeled CA1 neuronal axons/terminals (Figures 2D and 2F). THC elicited CB-LTD at CA3-CA1 synapses in tamoxifen-treated wild-type mice but not in GFAP-*CB$_1$R*-KO mutant littermates (Figures 2B and 2C). Therefore, cannabinoid exposure in vivo elicits CB-LTD at CA3-CA1 synapses through CB$_1$R expressed in astroglial cells.

Mechanisms of CB-LTD at CA3-CA1 Synapses

Cannabinoids are able to activate hippocampal astroglial CB$_1$R to increase extracellular glutamate levels (Navarrete and Araque, 2008). If a similar mechanism is involved in CB-LTD, LTD should be induced by the glutamate-uptake inhibitor DL-threo-β-benzyloxyaspartate (TBOA). Indeed, an i.c.v. injection of TBOA (10 nmol) (Wong et al., 2007) induced in vivo LTD at CA3-CA1 synapses (Figures 3A and 3F). If increased extracellular levels of glutamate induce LTD at CA3-CA1 synapse, postsynaptic metabotropic glutamate receptor (mGluR) may be responsible for this LTD induction, because postsynaptic mGluR activation produces LTD (Chevaleyre et al., 2006; Lovinger, 2008). However, the selective group I/group II mGluR antagonist ethyl-4-carboxyphenylglycine (E4CPG, 35 nM/3.5 μl, i.c.v.) completely blocked in vivo LTD induced by the group I mGluR agonist dihydroxyphenylglycine (DHPG, 100 nM/5 μl, i.c.v.), but did not alter CB-LTD (Figures 3B, 3C, and 3F). Surprisingly, CB-LTD was fully blocked by the selective NMDAR antagonist AP-5 (50 mM, intra-CA1 iontophoretic ejection at −20 nA for 10 min) (Maalouf et al., 1998) (Figures 3D and 3F), and by the NR2B-preferring NMDAR antagonists Ro25-6981 (6 mg/kg, i.p.) (Fox et al., 2006) and ifenprodil (5 mg/kg, i.p.) (Higgins et al., 2005) (Figures 3E and 3F). However, the NR2A-preferring NMDAR antagonist NVP-AAM077 (1.2 mg/kg, i.p.) (Fox et al., 2006) did not alter CB-LTD in the same conditions (Figures 3E and 3F). Thus, in vivo cannabinoid exposure induces CB-LTD at CA3-CA1 synapses via activation of NR2B-containing NMDAR.

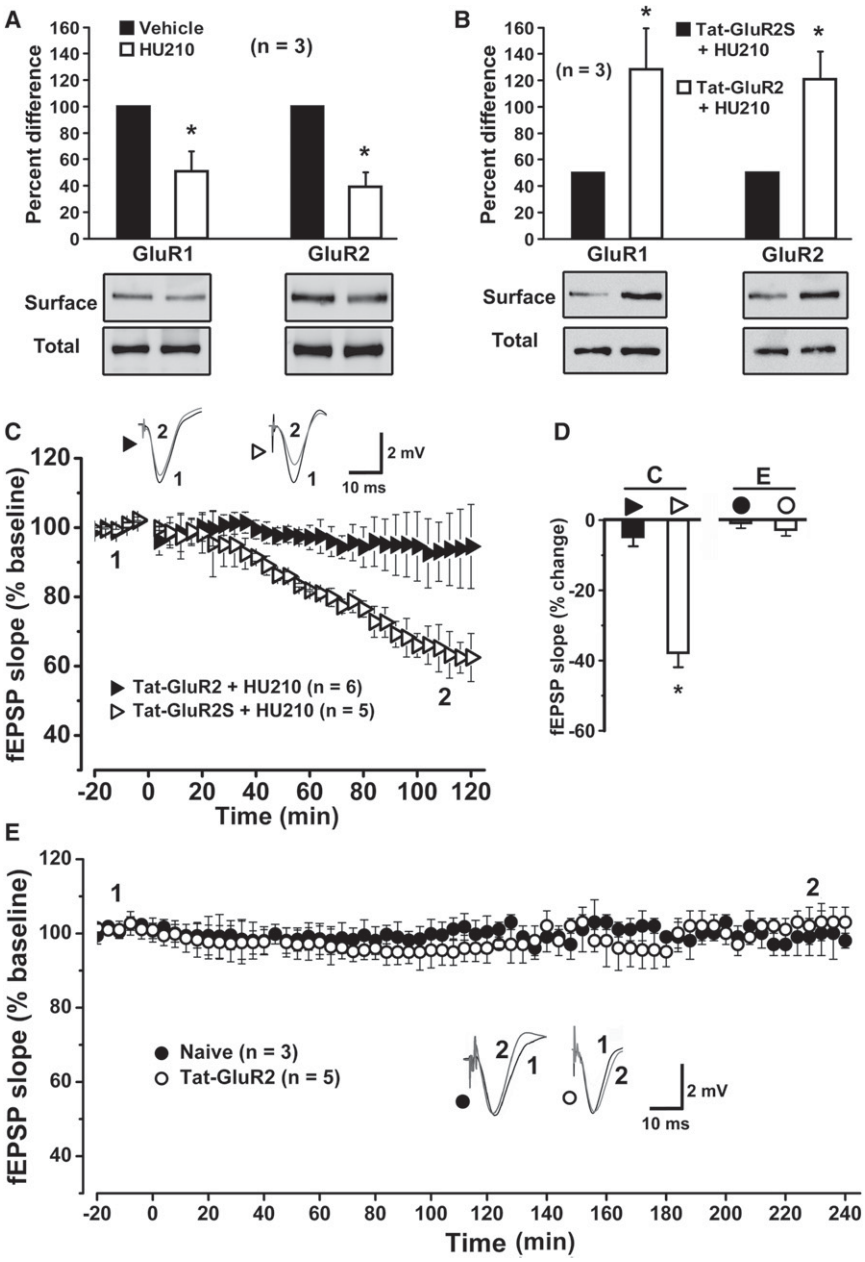

Figure 4. Cannabinoids Induce AMPAR Endocytosis-Dependent Expression of CA1 LTD

(A and B) Graphs and immunoblotting (bottom photos) show a decrease of GluR1 and GluR2 at the synaptic surface of CA1 neurons after HU210 injection, which is blocked by pretreatment with Tat-GluR2 but not Tat-GluR2S.

(C) Plot of normalized fEPSP slopes in anesthetized rats shows that injection of Tat-GluR2, but not Tat-GluR2S, 2 hr before HU210 injection at 0 min blocks HU210-induced LTD. Representative fEPSP traces before (1) and after (2) HU210 injection are shown above the plot.

(D) Histogram summarizes the average percent change of fEPSP slope before (1) and after (2) HU210 injection (C) or Tat-GluR2 injection (E).

(E) Plot of normalized slopes of fEPSPs in anesthetized rats shows both naive rats and rats receiving Tat-GluR2 injection at 0 min display similar fEPSPs at CA3-CA1 synapses for 4 hr. Representative fEPSP traces recorded during −10–0 min (1) and 230–240 min (2) are shown below the slopes.

All summary graphs show means ± SEM; n = numbers of experiments conducted (A and B) or numbers of animals recorded (C and E) in each group. *$p < 0.05$ versus control, t test.

The expression of NMDAR-mediated LTD requires facilitated endocytosis of postsynaptic AMPAR (Collingridge et al., 2010). AMPAR in CA1 pyramidal cells consists of 81% of GluR1/GluR2 at synaptic membranes (Lu et al., 2009). The surface levels of GluR1/GluR2 in synaptosomes isolated from the CA1 region significantly decreased after HU210 injection (Figure 4A), suggesting endocytosis of AMPAR in postsynaptic CA1 pyramidal cells following cannabinoid exposure in vivo. The administration of the brain-penetrating version of a peptide able to block GluR2 endocytosis (“Tat-GluR2” peptide, 1.5 μmol/kg, i.p.), but not of its scrambled analog (Tat-GluR2S) (Brebner et al., 2005; Wong et al., 2007; Collingridge et al., 2010), specifically blocked both HU210-induced GluR1/GluR2 endocytosis (Figure 4B) in the CA1 and CB-LTD (Figures 4C and 4D). Tat-GluR2 (1.5 μmol/kg, i.p.) did not significantly change the fEPSP amplitude at CA3-CA1 synapses for 4 hr after injection (Figures 4D and 4E). Altogether, these data strongly suggest that postsynaptic endocytosis of GluR1/GluR2 mediates the expression of CB-LTD at CA3-CA1 synapses.

Cannabinoid Impairment of Working Memory Shares the Same Mechanisms of CB-LTD

CB-LTD is characterized by (1) activation of astroglial CB_1R, (2) activation of NMDAR, and (3) internalization of AMPAR. These mechanisms were assessed in different behavioral models of cannabinoid impairment of spatial working memory (SWM).

The role of astroglial CB_1R in cannabinoid impairment of SWM was assessed by examining SWM performance of tamoxifen-treated GFAP-*CB_1R*-WT and GFAP-*CB_1R*-KO littermates with a delayed-matching-to place (DMTP) version of the Morris water maze test (Steele and Morris, 1999). No significant differences were observed between wild-type and mutant littermates during training (Figure S2A). In agreement with a previous study (Varvel and Lichtman, 2002), THC impaired SWM performance in GFAP-*CB_1R*-WT mice, as evidenced by a significant decrease of both latency saving ratios (Figure 5A) and path saving ratios (Figure 5B). In contrast, THC did not

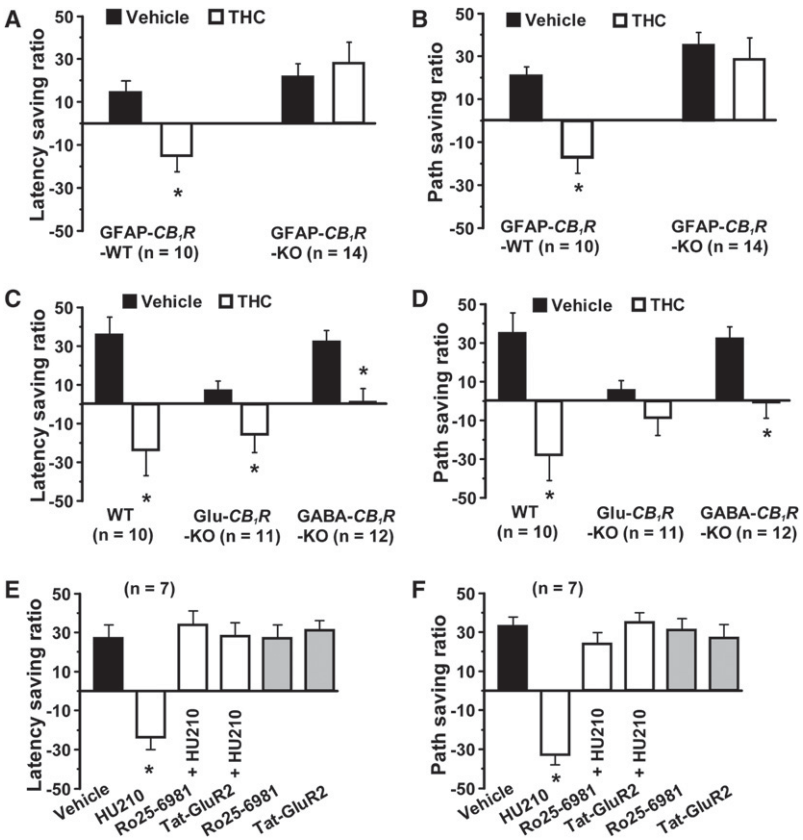

Figure 5. Astroglial CB_1R, NMDAR, and AMPAR Mediate Cannabinoid Impairment of SWM

(A–D) Mouse DMTP version of the Morris water maze test. THC reduces both latency saving ratio (A) and path saving ratio (B) in wild-type mice (A – D) and GABA-CB_1R-KO littermates but not in GFAP-CB_1R-KO littermates. While vehicle-treated Glu-CB_1R-KO littermates show a significant decrease of both latency saving ratio and path saving ratio relative to vehicle-treated wild-type mice (C and D), THC reduces latency saving ratio (C) but not path saving ratio (D) in Glu-CB_1R-KO littermates.

(E and F) Rat DMTP version of the Morris water maze test. HU210 reduces both path saving ratio (E) and latency saving ratio (F), which are prevented by i.p. pretreatment with Ro25-6981 or Tat-GluR2, while neither Ro25-6981 nor Tat-GluR2 significantly affects the ratio in the absence of HU210.

All summary graphs show means ± SEM; n = numbers of animals tested in each group. *p < 0.05 versus control, Bonferronni post-hoc test after repeated-measure two-way ANOVA ([A] $F_{1,22} = 13.010$, $p < 0.01$; [B] $F_{1,22} = 7.999$, $p < 0.01$; [C] treatment: $F_{1,30} = 37.28$, $p < 0.001$; genotype x treatment $F_{2,30} = 2.92$, $p > 0.05$; [D] treatment: $F_{1,30} = 30.01$, $p < 0.001$; genotype x treatment $F_{2,30} = 4.25$, $p < 0.05$) or one-way ANOVA ([E] $F_{5,36} = 19.307$, $p < 0.01$; [F] $F_{5,36} = 13.110$, $p < 0.01$).

See also Figures S2 and S5.

produce significant effects on GFAP-CB_1R-KO littermates (Figures 5A and 5B). While Glu-CB_1R-KO littermates showed a significant impairment of the acquisition of SWM (Figure S2B) and subsequent poor performance of SWM in comparison with wild-type mice (Figures 5C and 5D), THC impaired SWM performance (Figure 5C). Both GABA-CB_1R-KO littermates and control wild-type mice showed similar acquisition of SWM (Figure S2B), and THC impaired SWM performance (Figures 5C and 5D). THC treatment did not alter swim speed of GFAP-CB_1R-WT and GFAP-CB_1R-KO mice (Figure S2C), but slightly decreased this parameter in Glu- and GABA-CB_1R-KO mice and WT littermates (Figure S2D). However, this slight effect was equal for all genotypes (Figure S2D) and was equally distributed among different trials (data not shown), thereby excluding its involvement in the altered SWM performance of the mice. Thus, CB_1R in glutamatergic neurons, but not CB_1R in GABAergic neurons or astroglial cells, is necessary for mice to acquire SWM. Notably, however, astroglial CB_1R, but not glutamatergic or GABAergic neuronal CB_1R, is necessary to produce the detrimental effects of THC on SWM.

To test if NMDAR activation plays a role in cannabinoid impairment of SWM, rats were tested in a T-maze using a delayed nonmatching to sample protocol (DNMTST) (Kelsey and Vargas, 1993). After 6 daily training sessions to ensure that the task was mastered (>80% correct choices, Figure S3A), rats received 2 daily test sessions 30 min after injection of HU210 or vehicle. Ten min before HU210 injection, rats were pretreated with Ro25-6981 or ifenprodil, two NR2B-preferring NMDAR antagonists, or NVP-AAM077, a NR2A-prefering NMDAR antagonist. The results show that NR2B- but not NR2A-preferring NMDAR antagonists abrogated HU210-induced impairment of SWM performance (Figure 6A). Thus, activation of NR2B-containing NMDAR is necessary for the cannabinoid-induced impairment of SWM.

The effects of the blockade of AMPAR internalization on cannabinoid-induced SWM impairment was also tested in the DNMTST paradigm. After 6 daily training sessions (Figure S3B), rats received Tat-GluR2 or Tat-GluR2S (1.5 μmol/kg, i.p.) (Brebner et al., 2005; Wong et al., 2007) 2 hr before HU210 injection on each of the two testing days. Tat-GluR2, but not Tat-GluR2S, abolished HU210 impairment of SWM performance (Figure 6B). To determine the specific role of the CA1 region, after 6 daily training sessions (Figure S3C), Tat-GluR2 or Tat-GluR2S was infused bilaterally within the dorsal CA1 region (15 pmol/per injection) (Brebner et al., 2005; Wong et al., 2007) (Figure 6C) 60 min before each HU210 injection on each testing day. Intra-CA1 infusion of Tat-GluR2, but not Tat-GluR2S, blocked HU210 impairment of SWM performance (Figure 6D). Neither systemic nor intra-CA1 administration of Tat-GluR2 significantly affected basal locomotor activity, anxiety level or motor balance (Figures S4A–S4E). Thus, AMPAR internalization in the CA1 hippocampal region is necessary for cannabinoid-induced alteration of SWM.

If intra-CA1 infusion of HU210 is able to induce CB-LTD at CA3-CA1 synapses (Figures S1A and S1C), a bilateral intra-CA1 infusion of HU210 should impair SWM. As expected, after six daily training sessions (Figure S3D), HU210 (0.1 μg/0.5 μl/side) impaired rat SWM performance (Figure 6E).

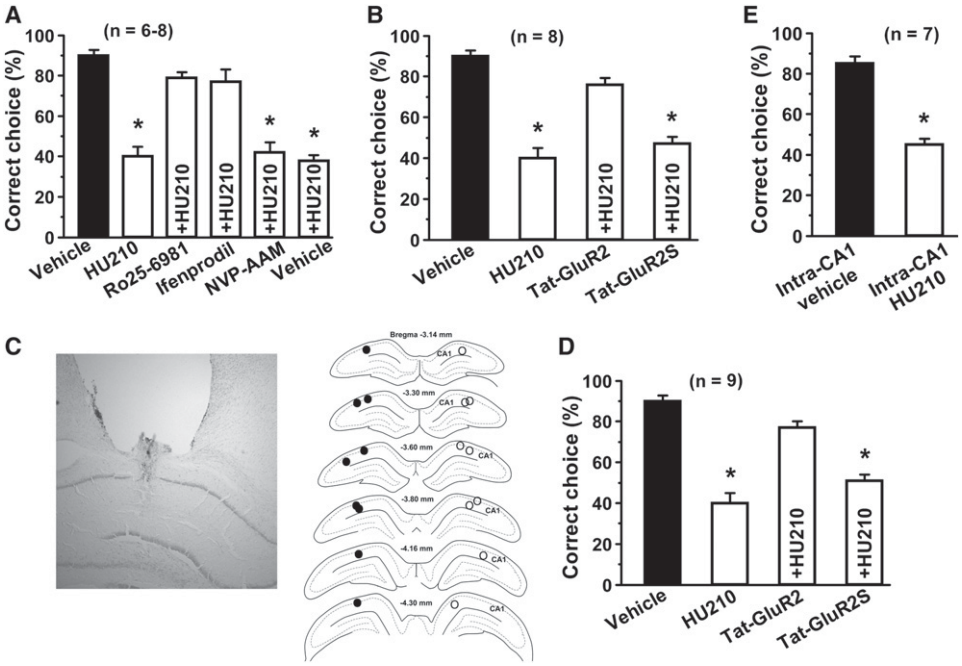

Figure 6. NMDAR and AMPAR Mediate Cannabinoid Impairment of SWM

(A) Rat DNMTS T-maze. HU210 suppresses SWM performances, which is prevented by i.p. pretreatment with Ro25-6981 and ifenprodil, but not with NVP-AAM077.

(B and D) Rat DNMTS T-maze. Systemic (B) and intra-CA1 administration (D) of Tat-GluR2, but not Tat-GluR2S, blocks HU210 impairment of SWM performance.

(C) Photograph (left) shows location of an intra-CA1 cannula, and histograms (right) show reconstructions of histology sections illustrating CA1 injection sites of Tat-GluR2 (solid circle) and Tat-GluR2S (open circle).

(E) Intra-CA1 injection of HU210, but not vehicle, impairs SWM performance.

All summary graphs show means ± SEM; n = numbers of animals tested in each group. *$p < 0.01$ versus control, Bonferronni post-hoc test after one-way ANOVA (A: $F_{5,36} = 59.070$, $p < 0.01$; B: $F_{3,28} = 54.220$, $p < 0.01$; D: $F_{3,32} = 41.562$, $p < 0.01$; E: $F_{1,12} = 36.090$, $p < 0.01$).

See also Figures S3 and S4.

Finally, we tested if the results obtained with the DNMTST paradigm were reproducible with the DMTP water maze paradigm. One day after five daily training sessions to establish the baseline levels of SWM (Figure S5A), rats received a test session of four trials. HU210 treatment before the test session impaired SWM performance, which was blocked by pretreatment with Ro25-6981 or Tat-GluR2 (Figures 5E and 5F). Neither Ro25-6981 nor Tat-GluR2 administration alone significantly changed saving ratios (Figures 5E and 5F), suggesting that neither NR2B-preferring NMDAR antagonists nor Tat-GluR2 interferes with basal SWM performance. Swim speeds during the SWM task were not influenced by different treatments (Figure S5B). Thus, cannabinoid administration alters SWM performance in different behavioral tasks through the same mechanisms.

Altogether, these data show that the same mechanisms underlying CB-LTD at hippocampal CA3-CA1 synapses (activation of astroglial CB_1R, activation of NMDAR and removal of AMPAR from the synaptic surface) also mediate cannabinoid-induced alterations of hippocampal-dependent SWM.

DISCUSSION

This study shows that one of the most common effects of cannabinoid intoxication in humans and animals, the impairment of SWM, is due to activation of astroglial CB_1R. Furthermore, a novel form of cannabinoid-induced long-term synaptic plasticity in the hippocampus appears to mechanistically underlie this effect of cannabinoids in vivo. Our results are consistent with a scenario (Figure 7), in which cannabinoid exposure in vivo activates astroglial CB_1R to increase ambient glutamate, which in turn activates NR2B-containing NMDAR to trigger AMPAR internalization at CA3-CA1 synapses. These events ultimately induce CB-LTD at these synapses, altering the function of hippocampal circuits that likely become unable to process SWM (Figure 7).

Early studies demonstrate that CB_1R is expressed at high levels by neurons throughout the whole brain (Herkenham et al., 1990; Matsuda et al., 1993; Tsou et al., 1998). More recent studies show that CB_1R is more abundant in GABAergic interneurons than in glutamatergic principal neurons (Kawamura et al., 2006). In the hippocampal CA1 area, CB_1R density on GABAergic presynaptic membranes is at least 10–20 times higher than that on glutamatergic presynaptic membranes (Kawamura et al., 2006; Bellocchio et al., 2010). Cannabinoid depression of in vitro excitatory or inhibitory synaptic transmission has been consistently shown to require CB_1R in either glutamatergic or GABAergic presynaptic terminals, respectively (Misner and Sullivan, 1999; Chevaleyre et al., 2006; Kawamura

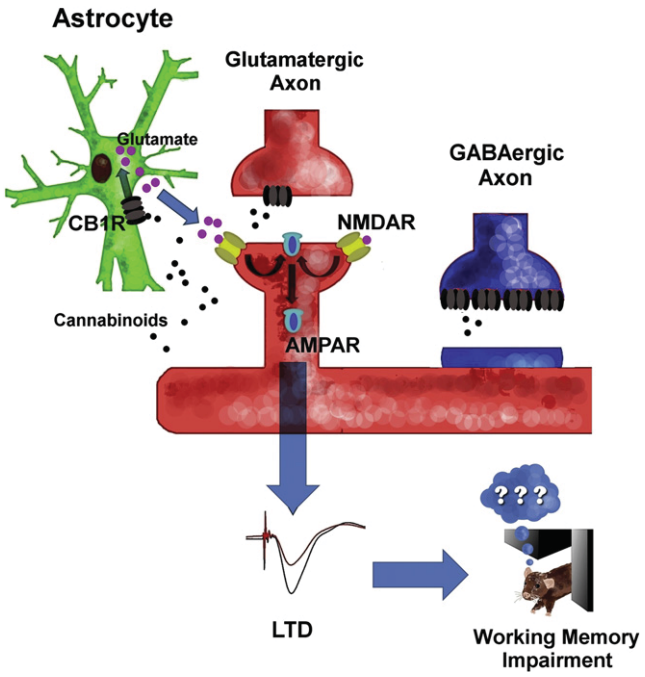

Figure 7. Proposed Model for In Vivo LTD Production at CA3-CA1 Synapses and Subsequent Working Memory Impairment

CB_1R exists in CA1 astrocytes (Figures 2D–2G) and presynaptic membranes with 10- to 20-fold of CB_1R density in GABAergic membranes than glutamatergic membranes (Kawamura et al., 2006). GABAergic and glutamatergic terminals containing CB_1R synapse with dendrites and spines of CA1 pyramidal cells, respectively (Kawamura et al., 2006). In vitro activation of presynaptic CB_1R by cannabinoids reduces the release of glutamate and GABA from glutamatergic and GABAergic membranes, respectively. However, cannabinoid exposure in vivo sequentially activates astroglial CB_1R and postsynaptic NR2B-containing NMDAR, which elicits AMPAR endocytosis-mediated expression of in vivo LTD at CA3-CA1 synapses, resulting in working memory impairment.

et al., 2006; Takahashi and Castillo, 2006; Navarrete and Araque, 2008, 2010; Bajo et al., 2009). Indeed, cannabinoids fail to reduce excitatory or inhibitory synaptic transmission in hippocampal slices of conditional mutant mice lacking CB_1R expression in either glutamatergic or GABAergic hippocampal neurons, respectively (Domenici et al., 2006; Monory et al., 2006). Unexpectedly, we observed here that in vivo exposure to exogenous cannabinoids induced full CB-LTD at excitatory CA3-CA1 synapses in both wild-type mice and mutant littermates lacking CB_1R in either CA1 glutamatergic or GABAergic neurons. These data do not support an involvement of glutamatergic or GABAergic CB_1R in in vivo CB-LTD at CA3-CA1 synapses.

The presence of CB_1R has also been suggested in brain astrocytes (Moldrich and Wenger, 2000; Rodriguez et al., 2001; Salio et al., 2002), but the extremely low levels of CB_1R expression in this cell population did not allow reaching the same conclusive evidence of functional data (Navarrete and Araque, 2008, 2010). The use of double immunostaining applied to wild-type and conditional or constitutive *CB_1R* mutant mice allowed us to provide conclusive electron microscopic evidence that CB_1R is expressed and quantifiable in hippocampal astrocytes. We have further showed here that in vivo CB-LTD at CA3-CA1 synapses was not detectable in tamoxifen-inducible conditional mutant mice specifically lacking CB_1R expression in astrocytes (i.e., GFAP-*CB_1R*-KO littermates). Our results strongly suggest a requirement of astroglial CB_1R for CB-LTD at CA3-CA1 synapses in living animals.

However, we also found that THC exposure in vivo did not significantly alter basal synaptic transmission in GFAP-*CB_1R*-KO littermates. These data, together with the finding that the density of presynaptic CB_1R at CA3-CA1 synapses is just above the background levels (Kawamura et al., 2006), suggest a negligible role of presynaptic CB_1R in excitatory transmission in vivo at CA3-CA1 synapses in response to exogenous cannabinoid exposure. Thus, in vitro cannabinoid application decreases excitatory synaptic transmission at CA3-CA1 synapses via activation of "glutamatergic" CB_1R, whereas in vivo cannabinoid administration induces CB-LTD via astroglial CB_1R without significant effects on presynaptic CB_1R. The exact reason for this apparent mechanistic discrepancy between in vitro and in vivo effects of cannabinoids on synaptic transmission and plasticity is not known. Nevertheless, it is important to note that intact astroglial networks play prominent roles in brain functioning (Giaume et al., 2010). Indeed, astrocytes are more associated in networks than neurons due to the presence of high levels of gap junctions and direct intercellular communications (Giaume et al., 2010). It is therefore possible that the unavoidable disruption of these networks by slicing procedures might alter the impact of astroglial CB_1R signaling in vitro. Meanwhile, slicing procedures might also upregulate the number or function of presynaptic CB_1R, leading to a decrease of glutamatergic transmission upon its activation by exogenous cannabinoids. This idea is supported by the evidence that although CB_1R density is at least 10-20 times higher on inhibitory than excitatory terminals in the CA1 region (Kawamura et al., 2006; Bellocchio et al., 2010), application of a saturating concentration of WIN22,212-2 (2 μM) to hippocampal slices produced similar depression (~50%) of EPSC (Kawamura et al., 2006) and IPSC (Hajos and Freund, 2002) in the CA1 area. Because brain slice preparations are extensively used for studying alterations of synaptic strength following in vitro application of other drugs of abuse, it is worthwhile to explore whether astrocytes play a key role in the in vivo effects of these drugs of abuse that are different from their in vitro effects.

Recent studies with brain slices show that endocannabinoids activate CA1 astroglial CB_1R to increase extracellular glutamate levels, which in turn activate presynaptic mGluR to induce LTP at CA3-CA1 synapses (Navarrete and Araque, 2008, 2010). However, we show here that cannabinoids activate astroglial cells to induce in vivo LTD at CA3-CA1 synapses. It is currently unknown why activation of astroglial CB_1R by in vitro endocannabinoid and in vivo cannabinoid induces, respectively, in vitro LTP and in vivo LTD at CA3-CA1 synapses. It is possible that activation of astroglial CB_1R in brain slices with disrupted astroglial networks might produce lower levels of interstitial glutamate than those produced in living animals with intact astroglial networks, which then activate presynaptic mGluR in vitro and

postsynaptic NMDAR in vivo, respectively, to induce in vitro LTP and in vivo LTD at CA3-CA1 synapses.

This study confirmed the consistent finding that HU210 and THC impair SWM in rodents (Lichtman and Martin, 1996; Hampson and Deadwyler, 2000; Nava et al., 2001; Varvel and Lichtman, 2002; Fadda et al., 2004; Hill et al., 2004; Wise et al., 2009). Although a recent study claimed the inability of systemic HU210 injection to impair SWM tested with the DMTP water maze paradigm (Robinson et al., 2007), this study failed to use the 'saving ratio' analysis as we and others (Varvel and Lichtman, 2002) have successfully used to identify the detrimental effects of HU210 and THC on rodent SWM performance.

While glutamatergic axonal CB_1R is in part responsible for cannabinoid-elicited locomotor suppression, catalepsy and hypothermia (Monory et al., 2007), hippocampal GABAergic axonal CB_1R likely plays a key role in cannabinoid impairment of long-term memory (Puighermanal et al., 2009). Our data using GABA-*CB_1R*-KO mice clearly show that "GABAergic" CB_1R is fully dispensable both for basal performance of the SWM task and, most importantly in this context, for the acute effect of exogenous cannabinoids. By showing that Glu-*CB_1R*-KO mice are impaired in basal performance of the SWM task, our data suggest that CB_1R expressed in cortical glutamatergic neurons participates in the endogenous control of SWM. This control might be exerted acutely by endogenous mobilization of endocannabinoids during the task or can also be due to developmental effects of *CB_1R* deletion in this cell population (Mulder et al., 2008). However, exogenous THC treatment of Glu-*CB_1R*-KO mice is still able to further reduce their poor performance, strongly suggesting the dispensable role of "glutamatergic" CB_1R in the acute effects of exogenous cannabinoids on SWM performance.

Conversely, by showing that GFAP-*CB_1R*-KO mice display normal learning of SWM, but totally fail to respond to THC, the present study provides striking evidence for the necessary role of astroglial CB_1R in SWM impairment induced by exogenous cannabinoids.

Cannabinoid-induced LTD and impairment of SWM share not only the dependency on astroglial CB_1R but also a whole series of well-defined molecular mechanisms. Thus, the pharmacological blockade of NR2B-containing NMDAR, but not NR2A-containing NMDAR, prevented both CB-LTD at CA3-CA1 synapses and cannabinoid impairment of SWM. Moreover, the Tat-GluR2 peptide can selectively block the facilitated endocytosis of AMPAR (Collingridge et al., 2010), the final step of the expression of NMDAR-dependent LTD (Collingridge et al., 2010), without significant effects on LTP induction or basal synaptic transmission (Collingridge et al., 2010). Both systemic and intra-CA1 application of the Tat-GluR2 peptide not only disrupted the expression of CB-LTD at CA3-CA1 synapses but also cannabinoid impairment of SWM, as assessed with both the DMTP version of the Morris water maze test and the DNMTS T-maze test.

Collectively, at least three key molecular mechanisms are shared by CB-LTD and cannabinoid-induced impairment of SWM: (1) activation of astroglial CB_1R by the exogenous cannabinoid; (2) increase of local glutamate and activation of NR2B-containing NMDAR; (3) endocytosis of AMPAR (Figure 7). These findings strongly suggest a causative role of CB-LTD at CA3-CA1 synapses in cannabinoid-induced impairment of SWM and reveal novel mechanistic views of the role of astrocytes in learning and memory processes and of the memory-disruptive effects of marijuana intoxication.

EXPERIMENTAL PROCEDURES

Generation of Mutant Mice

Constitutive *CB_1R*-KO mice and conditional Glu-*CB_1R*-KO and GABA-*CB_1R*-KO mice were generated and genotyped as described (Marsicano et al., 2002; Monory et al., 2006). GFAP-*CB_1R*-KO mice were generated using the Cre/loxP system. Mice carrying the "floxed" *CB_1R* gene (*$CB_1^{f/f}$*) (Marsicano et al., 2003) were crossed with GFAP-CreERT2 mice (Hirrlinger et al., 2006), using a three-step backcrossing procedure to obtain *$CB_1R^{f/f;GFAP\text{-}CreERT2}$* and *$CB_1R^{f/f}$* littermates, called GFAP-*CB_1R*-KO and GFAP-*CB_1R*-WT, respectively.

Immunohistochemistry for Electron Microscopy

Animals were transcardially fixed with 0.1% glutaraldehyde, 4% formaldehyde and 0.2% picric acid or with 2% formaldehyde and 8% picric acid. Hippocampal vibrosections were cut for double preembedding staining of CB_1R and GFAP with silver-intensified immunogold method and immunoperoxidase method. Tissue preparations were photographed for quantification of positive immunoreactive profiles. Detailed procedures are described in the extended methods in the SOMs.

Adenovirus Preparation and Administration

Recombinant adenoviruses were prepared as described (Liu et al., 2010). After intra-CA1 infusion of adenoviral vectors (10^{10} plaque-forming units/μl/injection), the CA1 area surrounding the injection tract was dissected 4 days later for quantification of CB_1R protein with procedures as described (Ji et al., 2006; Liu et al., 2010).

Synaptosomal Surface AMPAR Measurement

Biotinylation experiments for the CA1 area on hippocampal slices were performed as described (Kim et al., 2007). Protein fractions were transferred onto nitrocellulose membranes, which were probed with primary antibodies to GluR1 (1:250, Millipore, Billerica, MA) or GluR2 (1:500, Millipore, Billerica, MA) overnight at 4°C. Bands were analyzed by densitometry, and receptor ratios for AMPAR subunits were determined by dividing the surface intensity by the total intensity.

Electrophysiology Analysis

Under anesthesia, rats or mice received implantation of stimulating and recording electrodes into the CA1 region. fEPSPs were evoked by applying single pulses of stimulation at 0.067 Hz. Stimulus pulse intensities were 20-60 nA with a duration of 500 μs. Spike2 software was utilized to record data. Procedures for fEPSP recordings from freely moving rats were generally similar to those from anaesthetized rats with the exception of allowing rats to recover for 2 weeks after surgery for electrode implantation. Detailed procedures are described in the extended methods in the SOMs.

Behavioral Tests

Water Maze Test

Mice were tested in a DMTP version of the Morris water maze paradigm (Steele and Morris, 1999). Briefly, after a habituation session of 3 trials without spatial cues, mice received daily training sessions of 4 trials each with the maximal escape latency of 60 s, and 30 min before each of the sessions 6 through 12 and before the 13th session, mice were treated with vehicle and THC (5 mg/kg, i.p.), respectively. Performances of individual SWMs were calculated using the "saving ratio" procedure (Varvel and Lichtman, 2002) and calculated as follows: *path saving ratio = (path-length $trial_1$ - path-length $trial_4$) / (path-length $trial_1$ + path-length $trial_4$); and latency saving ratio = (escape latency $trial_1$ - escape latency $trial_4$) / (escape latency $trial_1$ + escape latency $trial_4$).* Procedures for rat water maze test were generally similar to mouse water maze test with the exception that rats received 5 daily sessions of SWM

training 1 day before a testing session of 4 trials with the maximal escape latency of 90 s. Detailed procedures are described in the extended methods in the SOMs.

Other Behavioral Tests

Rats were examined with the DNMTS T-maze test (Kelsey and Vargas, 1993), locomotor activity test (Ji et al., 2006), elevated-plus-maze test (Ji et al., 2006) and motor balance tests (Ji et al., 2006).

Statistical Analysis

Results were reported as mean ± SEM. Statistical analysis of the data was performed using a student t test, square Chi test, one-way ANOVA, or one-way or two-way ANOVA for repeated-measures, followed by Bonferronni post-hoc test. Statistical significance was set at $p < 0.05$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and five figures and can be found with this article online at doi:10.1016/j.cell.2012.01.037.

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EXHIBIT H

Neuropsychological deficits in long-term frequent cannabis users

Abstract—The authors examined neuropsychological functioning in 20 long-term (LT), 20 shorter term (ST) heavy frequent cannabis users, and 24 controls after abstinence for ≥24 hours prior to testing. LT users performed significantly worse on verbal memory and psychomotor speed. LT and ST users had a higher proportion of deficits on verbal fluency, verbal memory, attention, and psychomotor speed. Specific cognitive domains appear to deteriorate with increasing years of heavy frequent cannabis use.

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The number of cannabis users in Greece has doubled in the past decade.[1] Due to the possible therapeutic use of cannabinoids, it is important to replicate and extend previous findings of cannabis use on cognitive functions. Although the intoxication effects of cannabis use are well documented,[1,2] the effects on cognition after frequent, long-term use remain inconclusive.[2] A recent well-controlled study failed to demonstrate consistent neuropsychological deficits in frequent long-term cannabis users after an abstinence period of 28 days.[3] Others have found neuropsychological deficits in long-term cannabis users after an abstinence period of between 12 and 24 hours[4] and persistent neurocognitive deficits in heavy cannabis users after 28 days of abstinence.[5] In this study, we examined whether cognitive functions differ in groups of heavy, frequent cannabis users with longer and shorter term use after an abstinence period of ≥24 hours prior to neuropsychological testing.

Methods. We recruited participants aged 17 to 49 years from the drug abuse treatment program offered at the Saint Nicholas Clinic in Athens, Greece, in three groups: 1) 20 current heavy long-term frequent cannabis users, 2) 20 current heavy shorter term frequent cannabis users, and 3) 24 control subjects reporting that they had used cannabis at least once, but no more than 20 times in their lives, and had not used cannabis in the past 2 years. All participants underwent a detailed interview before entry into the study. Participants included reported regular cannabis use for at least 5 years, were currently smoking cannabis at least 4 days per week, and provided written consent for participation in the study. Our threshold for heavy frequent long-term cannabis use was four or more joints per week for at least 10 years (table 1). We excluded participants who reported 1) use or abuse of any other class of drugs (e.g., opiates, cocaine, stimulants) for more than 3 months throughout their lives and had used any of these drugs in the past year prior to participation in the study or 2) met a current *Diagnostic and Statistical Manual, 4th Edition* (DSM-IV) diagnosis of dependence on any other drug or alcohol (except cannabis); 3) met a current DSM-IV Axis I disorder; 4) current use of any psychoactive medication that may affect cognitive performance; 5) any other medical condition that might affect neuropsychological performance; 6) non-native speakers of the Greek language. Participants provided urine samples after at least 24 hours (range 36 to 240) of abstinence from cannabis use and another during the testing session. A urinary toxicology screen further confirmed that no other illicit substances were being used by the participants. We than administered a brief battery of neuropsychological tests to assess a range of cognitive abilities found in previous studies[3-5] to be affected by chronic and heavy cannabis use. All neuropsychological tests were administered using standard procedures in single sessions (table 2). Test results were analyzed with a series of analyses of variance, controlling for confounding variables that might affect neuropsychological performance (age, education level, estimated premorbid IQ, sex, severity of depression) through analyses of covariance.

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the March 14 issue to find the title link for this article.

Results. Initial analyses revealed differences between the groups for the confounding variables age ($F_{2,61}$ = 7.315; p = 0.001) and estimated premorbid IQ ($F_{2,61}$ = 6.052; p = 0.004). However, there were no differences between the groups as regards years of education ($F_{2,61}$ = 0.448; p = 0.641), severity of depression ($F_{2,61}$ = 0.141, p = 0.869), sex (proportion males/females) (χ^2_2 = 1.365; p = 0.505) and length of abstinence (t = 0.143; p = 0.887) (table 1).

Further analyses showed a group effect on all the trials of the Rey Auditory Verbal Learning Test (RAVLT) except trial 1 ($F_{2,59}$ = 0.115, p = 0.891) and trial 3 ($F_{2,59}$ = 0.115, p = 0.891). The learning curves of long-term (LT) and shorter term (ST) users were similar with post hoc multiple comparisons showing a difference between LT and ST users only on learning trial 2 of the RAVLT (p = 0.043). However, the LT group recalled fewer words on total trials 1 to 5 and delayed recall and recognition trials of the RAVLT. Post hoc multiple comparisons indicated differences between LT group and controls on trial 2 (p = 0.023), trial 4 (p = 0.016), trial 5 (p = 0.002), trial 6 (p = 0.000), delayed recall (p = 0.000), recognition (p = 0.015), and total trials 1 to 5 (p = 0.006) of the RAVLT. Differences were also found between ST and control groups on trial 5 (p = 0.008), trial 6 (p = 0.000), and delayed recall (p = 0.005) and recognition (p = 0.012) trial of the RAVLT. Analysis of covariance controlling for age and IQ showed a

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Table 1 *Demographic variables and cannabis use features of the sample (means, SD, and ranges)*

Variable	Total no. of cannabis users	Shorter term cannabis users (ST)	Long-term cannabis users (LT)	Control group
No.	40	20	20	24
Sex, M (%)	25 (62.5)	14 (70.0)	11 (55.0)	13 (54.2)
Age, y				
Mean (SD)*	28.45 (6.74)	24.25 (2.83)	32.65 (6.93)	28.42 (9.04)
Range	21–49	21–33	24–49	17–48
Years of education				
Mean (SD)	10.58 (2.59)	10.80 (2.21)	10.35 (2.96)	11.17 (3.20)
Range	6–16	6–16	6–16	6–20
Estimated IQ, mean (SD)	101.30 (5.72)	101.10 (5.90)	101.70 (5.40)	104.80 (4.30)
Duration of cannabis use				
Mean (SD)	11.28 (5.62)	6.95 (1.50)	15.60 (4.81)	—
Range	5–25	5–9	10–25	
Frequency of cannabis use				
Mean (SD)	20.43 (3.15)	20.70 (3.40)	20.15 (2.92)	—
Range, d/mo	16–28	16–28	16–28	
Length of abstinence				
Mean (SD)	124.55 (76.36)	122.80 (76.32)	126.30 (78.33)	—
Range, h	36–240	36–240	36–240	

Premorbid intelligence (IQ) was estimated by administering the vocabulary and matrix reasoning subscales of the Wechsler Abbreviated Scale of Intelligence, Gr- adapted version.[1] The vocabulary subscale is a good measure of crystallized intelligence, correlates well with general intellectual ability, and is relatively insensitive to cortical insults (i.e., a good measure of premorbid intellectual ability). The matrix reasoning subscale is a measure of nonverbal fluid reasoning and correlates well with general intellectual ability. These two subscales yield an estimated full-scale IQ.

* Significant at the $p < 0.05$ level; all other comparisons were not significantly different.

group effect for the Trail Making Test (TMT) Part A (TMT-A) ($F_{2,55} = 9.031$; $p < 0.001$) and Part B (TMT-B) ($F_{2,58} = 7.915$; $p = 0.001$). Post hoc multiple comparisons indicated differences between the LT group and controls on the TMT-A ($p = 0.036$) and TMT-B ($p = 0.011$). Differences were also found between ST group and controls on the TMT-A ($p < 0.001$) and TMT-B ($p < 0.001$). Analyses of covariance further indicated a group effect for phonemic fluency ($F_{2,59} = 13.100$; $p = 0.000$) and semantic fluency ($F_{2,59} = 16.908$; $p = 0.000$). Post hoc multiple comparisons showed that the LT group had worse performance on phonemic fluency ($p = 0.002$) and semantic fluency ($p = 0.000$) than the controls. Differences were also found between ST group and controls on phonemic fluency ($p < 0.001$) and semantic fluency ($p = 0.004$). A group effect was also found for the Boston Naming Test (BNT) ($F_{2,59} = 5.018$; $p = 0.01$). Post hoc multiple comparisons showed that only the LT group had a worse performance on the BNT than the controls ($p = 0.008$). (table 3).

Table 2 *Neuropsychological test battery arranged by cognitive function assessed*

Cognitive function(s) assessed	Test used
Verbal fluency/language	Boston Naming Test[8]
	Verbal fluency test: phonemic and semantic fluency[7]
Verbal memory/learning	Rey Auditory Verbal learning Test[9]
Psychomotor speed/ attention	Trail Making Test Part A[6]
Executive functioning	Trail Making Test Part B[6]
Severity of depression	Beck Depression Inventory–Fast Screen[10]

Normative data were taken from the sources indicated.

We also recorded the proportion of impairment on individual neuropsychological measures using two different criteria for impairment (1 and 1.5 SD below the control group mean). There were several different patterns in the proportion of impairments seen across the groups (see tables E-1 and E-2 on the *Neurology* Web site at www.neurology.org). We found a steady increase in the proportion of subjects classified as impaired, with the lowest rates in the control group and the highest in the LT group. The correlation of duration of cannabis use and neuropsychological measures for collapsed cannabis users (n = 40) revealed significant negative correlations between trials 2, 6, delayed recall, total trials 1 to 5 of the RAVLT, semantic fluency, BNT, and years of cannabis use (see table E-3). Duration of cannabis use therefore appears to be related to neuropsychological performance in certain cognitive domains.

Discussion. We investigated the chronic effects of frequent heavy cannabis use on cognitive functions, with duration of use as our main variable.

Table 3 *Neuropsychological test performance of long- and shorter term cannabis users and normal controls: Mean (SD)*

				p Value for comparisons		
	Shorter term cannabis users	Long-term cannabis users	Control group	Shorter term vs long term	Long term vs control	Shorter term vs control
RAVLT						
Trial 1	7.45 (2.46)	6.85 (1.53)	7.92 (1.74)	>0.99	>0.99	>0.99
Trial 2	9.95 (1.96)	7.70 (1.45)	10.04 (1.78)	0.043*	0.023*	>0.99
Trial 3	10.35 (1.48)	9.45 (1.10)	11.88 (2.01)	>0.99	0.057	0.065
Trial 4	11.30 (2.30)	10.20 (3.01)	12.71 (2.12)	>0.99	0.016*	0.073
Trial 5	11.50 (1.93)	10.25 (1.97)	13.17 (1.58)	>0.99	0.002*	0.008*
Trial 6	8.30 (2.54)	7.25 (1.86)	11.38 (2.06)	>0.99	<0.001*	0.000*
Delayed recall	9.30 (3.59)	6.60 (2.52)	12.17 (2.35)	0.401	<0.001*	0.005*
Recognition	11.30 (3.11)	11.20 (1.99)	13.75 (1.70)	>0.99	0.015*	0.012*
Total on trials (1-5)	50.50 (9.67)	42.65 (7.92)	55.29 (7.54)	0.404	0.006*	0.273
Semantic fluency	48.35 (7.95)	38.40 (6.85)	57.17 (9.74)	0.063	<0.001*	0.004*
Phonemic fluency	27.35 (9.25)	28.20 (4.60)	40.67 (9.18)	>0.99	0.002*	<0.001*
TMT-A (s)	53.15 (16.52)	52.79 (13.89)	34.67 (9.31)	0.104	0.036*	<0.001*
TMT-B (s)	100.42 (39.87)	107.55 (37.60)	65.25 (14.40)	0.332	0.011*	<0.001*
BNT	14.35 (0.75)	13.45 (1.00)	14.67 (0.64)	0.102	0.008*	>0.99
BDI–Fast Screen	7.85 (3.87)	7.84 (4.22)	7.33 (3.27)	>0.99	>0.99	>0.99

* Significant at the $p < 0.05$ level; all other comparisons were not significantly different.

For the TMT-A, the analysis was performed on 60 subjects due to the removal of one outlier case from the long-term users and three outlier cases from the controls. For the TMT-B, the analysis was performed on 63 subjects due to the removal of one extreme outlier case from the long-term users.RAVLT = Rey Auditory Verbal Learning Test; TMT-A = Trail Making Test Part A; TMT-B = Trail Making Test Part B; BNT = Boston Naming Test; BDI = Beck Depression Inventory.

By requiring an abstinence period of ≥24 hours prior to neuropsychological testing, we simulated an unintoxicated cognitive state in which LT users typically operate for substantial periods in their life. LT users performed significantly poorer on verbal memory vs ST users and controls. LT and ST users generated fewer words and demonstrated higher impairment rates than controls on both phonemic and semantic fluency. LT and ST users showed inferior performance vs the control subjects on psychomotor speed, attention, and executive functions. The greatest deficits regarding the LT users were seen on almost every trial of the RAVLT, indicating a generalized verbal memory deficit with impaired verbal learning, retention, and retrieval. LT users' performance was significantly poorer than the published norms (table 2) on most measures of the RAVLT. Our findings are in accordance with certain studies[4,5] showing that heavy long-term frequent cannabis use leads to subtle deficits in specific neuropsychological domains.

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EXHIBIT I

THIRD JUDICIAL DISTRICT DEPARTMENT OF CORRECTIONAL SERVICES

PROBATION AGREEMENT

I, Troy Kunkel #6381908, have been granted probation for the following offense(s), with further order of the Court being that I be placed under the supervision of the Judicial District Department of Correctional Services, as provided by Chapter 907, Code of Iowa.

Jurisdiction-Cause: Woodbury-SRCR062687 **Count:** 1
Offense Description: CHILD ENDANGERMENT/NO INJURY
Class: Aggravated Misdemeanor
Supervision Discharge Date: 06/30/2006
Sentence Disposition: Original Sentence **Sentence Date:** 06/30/2004
Penalty Type - Modifier: Prison - Suspended-With Probation **Value:** 2 yrs, 0 mths, 0 days
Penalty Type - Modifier: Fine - Suspended-No Probation **Value:** 500
Penalty Type - Modifier: Community Service - Imposed **Value:** 20

I, Troy Kunkel, do hereby agree that I will be subject to the following rules.

1 I shall obey all laws, whether they be federal or state laws or city ordinances.

2 I will contact my supervising officer within 24 hours upon any arrest or citation.

3 I shall be restricted to my county of residence unless prior permission to travel has been granted by my supervising officer or otherwise in accordance with the probation agreement. I shall secure advance written permission from my supervising officer before traveling outside of my state of residence. (I must notify my supervising officer 5 days in advance in order to secure permission for out of state travel.)

4 I shall secure and maintain employment as approved by my supervising officer. I shall obtain advanced permission from my supervising officer before changing or quitting a job. If I am fired or laid off, I shall notify my supervising officer within 24 hours. If I am/become unemployed, every effort shall be made to obtain employment, and such efforts shall be reported to my supervising officer as directed.

5 I shall obtain prior permission from my supervising officer before changing residency or telephone numbers.

6 I will report to my supervising officer in person as directed, and shall not lie to, mislead, or misinform my supervising officer either by statement or omission of information. I will submit a truthful monthly report by the 10th of each month.

7 I agree to participate in any treatment/rehabilitative/monitoring program directed by my supervising officer.

8 I shall maintain and, upon request, present proof of adequate liability insurance or proof of financial responsibility and a valid driver's license before owning or operating a motor vehicle.

9 I shall (abstain from) (~~limit~~) the use of alcoholic beverages. I shall not use any drugs unless prescribed for me by a licensed physician and will not use or possess any illegal or prescription drugs for which I do not have a valid prescription. I agree to submit to a urinalysis and/or alcohol testing upon the request of my supervising officer.

10 If on probation for a felony offense, or aggravated misdemeanor involving firearms or explosive devices, I will not own, possess, use, or transport firearms or other dangerous weapons. (Note: both federal and Iowa law prohibit a felon from possessing, using, or transporting a firearm. Also, federal law prohibits a person convicted of any misdemeanor domestic violence crime from possessing a firearm.)

11 I will pay to the supervising District Department of Correctional Services an enrollment fee of $250.00 to offset the costs of my supervision as provided in Section 905.14 of the Iowa Code. I will pay this fee upon such terms as my supervising officer directs. I understand that I will not be discharged from probation until this fee is paid.

12 I will pay all Court costs, restitution, and attorney fees as ordered by the Court, at the rate set in the Plan of Payment.

SPECIAL CONDITIONS:

Special Conditions - None

CASE MANAGER RULES:

S01. I shall obtain a substance abuse and/or mental health evaluation and follow all recommendations during supervision.

S02. I shall complete Anger Management training.

S03. I shall support my dependents and meet other family responsibilities.

S04. I shall work regularly at least 30 hours at a lawful occupation unless excused by my Probation Officer for schooling, training, or other acceptable reasons.

S05. I shall not frequent places where controlled substances are illegally sold, used, distributed, or administered.

S06. I shall notify my Probation Officer of any material change in my economic circumstances that would prohibit my ability to pay any unpaid restitution, fines, child support, or any other obligations until such obligations are paid in full.

S07. I shall reside in a community treatment center, halfway house, or Residential Treatment Facility or similar facility. This requirement may be waived solely at my Probation Officer's discretion.

S08. I shall perform 20 hours per week of community service at a place approved by my Probation Officer and provide proof of completion if not employed by 10-4-2004.

S09. I shall reside with my parents, James and Deborah Kunkel, unless granted permission by my Probation Officer.

S10. I shall enroll in and successfully complete Project ~~Compass~~ Phoenix.

I understand that my failure to comply with the above will be deemed to be a violation of the terms and conditions of probation, for which my probation may be revoked by the Court or in my being transferred to a more restrictive level of the department's corrections continuum, including, but not limited to, placement in a residential treatment facility.

Entering into any informant-type activity with any law enforcement agency will not excuse liability for any violation of my probation supervision. I hereby waive extradition from any State or County to the State of Iowa if I am arrested for an Iowa probation violation warrant. I understand that I may file a formal grievance through the established Departmental Grievance Procedures against actions of the Department.

I hereby certify that I have read (or had read to me) the above agreement, and that I do understand and agree that it shall be in full force and effect until I have received my final discharge from probation. I further certify that I have received a copy of this probation agreement.

Signed and witnessed this 16th day of July, 2004.

Troy Kunkel
Probationer

Probation/Parole Officer

Distribution: Officer (original), Client, Clerk of Court

EXHIBIT J

DEPARTMENT OF CORRECTIONAL SERVICES
THIRD JUDICIAL DISTRICT
RESIDENTIAL TREATMENT FACILITY
515 WATER STREET
SIOUX CITY, IA 51103
712-252-3451 Fax: 712-252-0634

October 15, 2004

Craig Hartman
Parole/Probation Officer
515 Water Street
Sioux City, IA 51103

SUBJECT OF REPORT: Termination from the Residential Treatment Facility

NAME: Troy David Kunkel
CAUSE #: Woodbury County, SRCR062687, Child Endangerment/No Injury
ADDRESS: Unknown
EMPLOYMENT: Unknown

IDENTIFICATION: On June 30, 2004 Troy Kunkel appeared in Woodbury County District Court for the offense of Child Endangerment/No Injury, Cause # SRCR062687. At that time he was sentenced to 2 years with the Department of Adult Corrections, suspended and was placed on two years probation to the Third Judicial District, Department of Correctional Services Sioux City, Iowa. Troy Kunkel was signed to the proper probation on July 16, 2004. Mr. Troy Kunkel did enter the Residential Treatment Facility on September 16, 2004 on probation status. On October 15, 2004, Mr. Troy Kunkel was terminated from the Residential Treatment Facility for escape. His whereabouts are currently unknown.

RESIDENT PROGRESS: Each client upon entering the Residential Treatment Facility is given a copy of the rules and regulations of the program. Additionally, two staff members on two separate occasions meet with the new client to review the rules and regulations and expectations of this program. Troy Kunkel met with the first staff member on September 21, 2004 and completed the first half of the intake process. Troy Kunkel then met with the second staff member on September 21, 2004, and completed the second part of the intake process. During the intake process, rule violations are defined and clarified for the resident. During Mr. Kunkel's 29 days in this program, he had eleven major violations. The violations are listed as follows:

August 28, 2004 – Major Violations – # 5 " False statements," # 24 " Fighting Assault," and # 28 " Obstructive/disruptive conduct." On 10/4/04 Residential Treatment Facility staff member Aslani wrote the following violation report: " On 10-3-04, approximately at 11:AM, resident Kunkel asked for an alcohol pad to clean his scratched sheen. I asked him what happened? Resident Kunkel stated, "I fell against a chair in the rec. room." I only suspected that the scratches were due to horseplay and decided not to charge Kunkel with any violation.

On 10-4-04, I read a report by RO Wyatt dated 10-3-04, stating that resident Kunkel had gone to Mercy Medical around 9:00PM for three fractured/injured ribs. Kunkel states to RO Wyatt that he injured himself in his room falling against his bed. I questioned resident Kunkel about the cause of his ribs injury. Kunkel admitted to this writer and RO Stacy that he suffered ribs injuries and the scratches when he was horse playing with another resident. Kunkel confessed that another resident jumped on his rib cage when he was lying down.
Because on 10-3-04, resident Kunkel lied to this RO when he stated that he scratched himself falling against a chair in rec. room, he is charged with first count of rule violation #5:False statements.
Because resident Kunkel told RO Wyatt that he injured his ribs in his room, he is charged with 2nd count of rule violation #5:False statements.
Because resident Kunkel engaged in horse playing that resulted in injury, he is charged with rule violation #24:Fighting/assault
Because resident Kunkel's conduct was disruptive, he is charged with rule violation #28:Obstructive/Disruptive conduct." The Disciplinary Hearing did meet on this matter on 10/6/04 at 4:09 AM and did find him guilty. The sanction imposed was seven additional weeks in Phase I.

October 11, 2004 – Major Violations – # 5 " False statements," and # 13 " Failing to secure/maintain employment." On 10/10/04 at 9:41 AM Residential Treatment Facility staff member Heilman wrote the following Disciplinary Report: " On 10/11/04 Resident Kunkel returned from work at 8:05 AM and he told RO Koll that he was let go from his job because he had too many appointments that were going to cause him to miss too much work. I called and spoke with April at Advance Services to verify this and she told me that she talked to Chris, Resident Kunkel's supervisor at Lite-Form, and he said that Resident Kunkel told him that he was quitting because he was too busy and had too many appointments.
Because Resident Kunkel told RO Koll he was let go from his job at Lite-Form when in fact he quit he is being charged with rule violation #5, "False Statements". Also because Resident Kunkel quit his job without permission from his counselor he is being charged with rule violation #13, "Failing to Secure or Maintain Employment". A Disciplinary Hearing was held on this matter on 10/13/04 at 6:27 AM. The sanction imposed was termination from the Residential Treatment Facility program.

October 11, 2004 – Major Violations # 1 " Illegal Behavior," # 5 " False Statements," and # 8 " Possession of drugs/intoxicants." Staff member Bill Wyatt wrote the following violation report on 10/11/04 at 8:07 PM: "On 10/11/04 this writer had Resident Kunkel to drop a UA and he tested positive for THC. While Resident Kunkel was dropping a UA, this writer asked him had he used any medication or drugs and he stated "NO." As a resident in the facility it is against the law to use drugs or any kind of mood altering drug. Because Resident Kunkel used Illegal drugs as a resident, he is being charged with a PB Violation #1 "Illegal Behavior." Because Resident Kunkel tested positive for THC, he is being charged with a PB Violation #8 "Possession Drugs/Intoxicant." Because Resident lied about not using any mood-altering drug (THC), he is being charged with a PB Violation #5 "False Statements." A Disciplinary Hearing was held on this matter on 10/13/04 at 6:04 AM. The sanction imposed was termination from the Residential Treatment Facility program.

September 13, 2004 – Major Violations – # 12 " Out of place of assignment," and # 11 " Escape." On 10/14/04 at 8:53 PM Residential Treatment Facility staff member Bill Burch wrote the following Disciplinary Report: " On 10/14/04 at 4:49 PM resident Kunkel signed out to JRC and Drilling Pharmacy. He said he had a 5:30 appointment at JRC with a return time of 7:20PM. At the time of this report 9:00 PM, he has not returned to the RTF and his whereabouts is unknown. As such he is charged with rule violation # 11 and # 12.He has been OPA for at least 4 hours."
The Disciplinary Hearing did meet on 10/15/04 at 7:26 AM and did find resident Kunkel guilty on both charges due to the fact he did escape and his whereabouts are currently unknown. The sanction imposed was termination from the Residential Treatment Facility program.

CASE PLANNING: Mr. Kunkel had a case plan of maintaining sobriety through the Jackson Recovery Center and following through on any recommended treatment. He was required to attend two AA/NA meetings per week. It should be noted Mr. Kunkel was involved in treatment at the Jackson Recovery Center Connections Group.. The fact he had a positive UA for THC and he chose to escape the Residential Treatment Facility program indicates he failed to comply with his case plan, probation agreement and substance abuse treatment requirements.

SUMMARY/RECOMMENDATIONS: In light of the fact Mr. Kunkel did escape from the Residential Treatment Facility and was officially terminated from the Residential Treatment Facility by actions he chose, this indicates he was not abiding by the rules and requirements that were set forth by his probation agreement, the Residential Treatment Facility as well as the orders set forth by the Woodbury County District Court. It is requested at this time that steps be taken by the Woodbury County Attorney's Office that a probation revocation proceeding be initiated citing Troy Kunkel for probation violation.

Submitted by:

Stan Orzechowski
Parole/Probation Officer

Approved by:

Steven Scholl
Division Manager

EXHIBIT K

JUDICIAL DISTRICT DEPARTMENT OF CORRECTIONAL SERVICES
REPORT OF VIOLATION

DATE: 10/18/2004

NAME: Troy David Kunkel

SSN: [redacted]

DOB: [redacted]

ADDRESS:
unknown,

Offense Date	Jurisdiction-Cause	Charge Count	TDD/SDD	Class
Offense Description				
Sentence Disposition Status	Sentence Date	End Date		
Penalty Type	Value	Modifier	Min Value	Max Value
12/22/2003	Woodbury-SRCR062687	1	06/30/2006	Aggravated Misdemeanor
726.6(6) (2001) -- CHILD ENDANGERMENT/NO INJURY				
Original Sentence	06/30/2004			
Prison	2, 0, 0	Suspended-With Probation	2, 0, 0	2, 0, 0
Fine	500	Suspended-No Probation		
Community Service	20	Imposed		

I, Craig Hartman, having been duly sworn, depose and say I verily believe Troy David Kunkel has violated his/her probation in the following violations, to wit:

VIOLATION(S)

RULE VIOLATED: S07. I shall reside in a community treatment center, halfway house, or Residential Treatment Facility or similar facility. This requirement may be waived solely at my Probation Officer's discretion.

INCIDENT DATE	VIOLATION COMMENTS
10/15/2004	Mr Kunkel. was terminated from the Residential Treatment Facility program in Sioux City on 10/15/04. Please review the attached report prepared by RTF counselor Stan Orzechowski for further details.

RULE VIOLATED: 5. I shall obtain prior permission from my supervising officer before changing residency or telephone numbers.

INCIDENT DATE	VIOLATION COMMENTS
10/15/2004	The defendant has not made his current whereabouts known to this officer since leaving the Sioux City RTF on 10/15/04.

RULE VIOLATED: 9. I shall (abstain from) (limit) the use of alcoholic beverages. I shall not use any drugs unless prescribed for me by a licensed physician and will not use or possess any illegal or prescription drugs for which I do not have a valid prescription. I agree to submit to a urinalysis and/or alcohol testing upon the request of my supervising officer.

INCIDENT DATE	VIOLATION COMMENTS
10/11/2004	On 10/11/04, Mr Kunkel submitted a urine sample to the RTF staff for the purpose of drug testing. The sample was positive for THC (marijuana).

COMMENTS/RECOMMENDATIONS:

This officer is recommending that Mr Kunkel be brought back before the Court to re-evaluate his probation status in Woodbury County once he is apprehended.

I certify under penalty of perjury and pursuant to the laws of the State of Iowa the preceeding is true and correct.

Respectfully submitted,

Craig Hartman
Probation/Parole Officer II

Jeffrey Page
Division Manager

Distribution: ~~Judge~~, County Attorney, ~~Defense Attorney, Offender~~, File

EXHIBIT L

IN THE IOWA DISTRICT COURT FOR WOODBURY COUNTY

THE STATE OF IOWA, '03 DEC 16 P1:17 Plaintiff, vs. TROY KUNKEL, Defendant.	SRCR062687 TRIAL INFORMATION

COMES NOW Jill R. Pitsenbarger, Assistant Woodbury County Attorney, and in the name and by the authority of the State of Iowa accuses Troy Kunkel of the crimes of:

Count 1: CHILD ENDANGERMENT RESULTING IN BODILY INJURY
Count 2: ASSAULT WHILE PARTICIPATING IN A FELONY

Committed as follows:

Count 1

Said defendant, during the time beginning on or about November 10, 2003 through the time ending on or about November 13, 2003, in Woodbury County, Iowa, who had custody or control over a child, knowingly acted in a manner that created a substantial risk to a child's physical, mental or emotional health or safety that resulted in bodily injury to the child, all in violation of Iowa Code Sections 726.6(1) and 726.6(5).

Count 2

Said defendant, during the time beginning on or about November 10, 2003 through the time ending on or about November 13, 2003, in Woodbury County, Iowa, assaulted another person while participating in a felony, all in violation of Iowa Code Sections 708.1 and 708.3.

A TRUE INFORMATION

Jill R. Pitsenbarger - WO0013040
Assistant Woodbury County Attorney

State of Iowa vs. Troy Kunkel
Trial Information

APPROVAL ORDER

This information and the minutes of testimony accompanying it have been examined by me and found to contain sufficient evidence, if unexplained, to warrant a conviction by a trial jury, the filing of this information is approved by me on this 16 day of December 2003.

_____ Bond is set in the amount of $_____ which shall be cash or approved surety only. The clerk of court shall issue an arrest warrant.

xxx Clerk of court shall transfer the court file to District Court. Bond previously set in this case number ($2,000 cash or approved surety only) shall continue as the bond set for the offenses charged in this trial information. The complaints and affidavits charging indictable offenses in this case number are dismissed. The preliminary hearings in this case number are cancelled.

_____ Bond previously set in this case number shall continue as the bond set for the offenses charged in this trial information. The complaints and affidavits charging indictable offenses in this case number are dismissed. The preliminary hearing in this case number is cancelled and the arraignment on the offenses charged in this trial information shall occur on the date and time previously set for preliminary hearing.

John D Lehman
Judge, 3rd Judicial District of Iowa

17433

EXHIBIT M

IN THE IOWA DISTRICT COURT FOR WOODBURY COUNTY

SRCR062687

THE STATE OF IOWA,)
CITY OF SIOUX CITY)
Plaintiff,)
vs.) COMPLAINT & AFFIDAVIT
)
TROY D. KUNKEL)
Defendant.)

THE DEFENDANT IS ACCUSED OF THE CRIME OF ASSAULT (SERIOUS), a SERIOUS MISDEMEANOR, in violation of Section 708.1(2) of the Iowa Criminal Code in that the Defendant on or about the 11th day of November, 2003, at SIOUX CITY, in Woodbury County, did:

COMMIT AN ACT WHICH WAS INTENDED TO PLACE ANOTHER IN FEAR OF IMMEDIATE PHYSICAL CONTACT WHICH WILL BE PAINFUL, INJURIOUS, INSULTING, OR OFFENSIVE, COUPLED WITH THE APPARENT ABILITY TO EXECUTE THE ACT, TO WIT:

THEREFORE, complainant requests that said Defendant, subject to bail or conditions of release where applicable, (1) be arrested or that other lawful steps be taken to obtain Defendant's appearance in court; or (2) be detained, if already in custody, pending further proceedings, and that said defendant otherwise be dealt with according to law.

S.C. P.D.
Complainant's Agency

J. KAYL #5224
Complainant

AFFIDAVIT

I, the undersigned Complainant, being first duly sworn on oath, do hereby depose and state I believe the above-named Defendant committed the above-named public offense based on the following facts known by me or told to me by other reliable persons:

DURING THE TIME FRAME OF 11/10/03 TO 11/13/03 AND AT THE LOCATION OF 2728 SO HELEN ST. APT. 23 THE ABOVE NAMED DEFENDANT WAS CARING FOR A 3 YR OLD MALE CHILD THAT RECIEVED NUMEROUS BRUISES TO HIS BODY AND A BROKEN RIGHT ARM. THE DEFENDANT INDICATED THAT HE STRUCK THE CHILD ON THE BUTTOCKS SEVERAL TIMES DURING A INCIDENT OF DISCIPLINE AND ANGER.

J. Kayl 5224
Complainant's Signature

SUBSCRIBED AND SWORN to before by the person signing this Complaint and Affidavit on this 9 day of December, 2003

Notary Public

APRIL A. MOORE
Commission Number 165869
My Commission Expires

I, the undersigned Judge have determined from the Complaint that there is probable cause to believe that the above-named Defendant committed the above-named public offense.

DATED this _____ day of _____, _____.

Judge

EXHIBIT N

FILED

IN THE IOWA DISTRICT COURT FOR WOODBURY COUNTY

'04 JUN 30 P1:13

CRAIG JACOBSEN
CLERK OF DISTRICT COURT

BY: _____ CLERK DESIGNEE

THE STATE OF IOWA,

Plaintiff,

vs.

Troy Kunkel

Defendant.

CRIMINAL NO. SRCR062687

SENTENCING ORDER

D/KB

Date: 6/30/2004

Defendant's current address: c/o jail

Defendant's date of birth: 4-19-83

Prosecuting Attorney: Pitsenbarger

Defendant's Attorney: Williams

Court Reporter: Early

Interpreter: _____

____ The State of Iowa moves to amend Count ___ of the Trial Information to charge the Defendant with _____, a(n) _____ Misdemeanor, in violation of Iowa Code section _____. The court grants the motion to amend.

This matter comes before the court for plea taking and sentencing. The Defendant has submitted a plea of guilty. The court finds that the Defendant's plea of guilty is voluntarily and intelligently made. The court accepts the Defendant's plea of guilty.

The Defendant stands convicted and is guilty of the crime of Child Endangerment _____, a(n) Aggravated Misdemeanor, in violation of Iowa Code section 726.6(6).

Sentence is as follows:

____ The State of Iowa does not resist a deferred judgment.

____ Judgment is deferred to _____.

X The Defendant is committed to the custody of the director of adult corrections for a term not to exceed two (2) years. This sentence of incarceration:

X Is suspended.

___ Is not suspended.

____ The Defendant is committed to the Woodbury County Jail for a period of _____ days. Of this sentence, _____ days are suspended.

____ Mittimus shall issue:

___ Forthwith.

___ On the _____ day of _____, 20____, at _____ ___.m.

___ MALES ONLY: The Defendant shall participate in the Sheriff's Weekend Work Program with mittimus to issue as follows: The Defendant shall report to the Prairie Hills Work Release Center for pre-booking between 6:00 a.m. and 6:00 p.m. on Friday, _____, 20___. After pre-booking, the Defendant shall be released, but the Defendant must return to the Prairie Hills Work Release Center the following morning, Saturday, _____, 20___, by 7:00 a.m. to begin serving the sentence. If successful in the program, the Defendant will be released at 5:00 p.m. Sunday.

___ FEMALES ONLY: The Defendant shall participate in the Sheriff's Weekend Work Program with mittimus to issue as follows: The Defendant shall report to the Prairie Hills Work Release Center for pre-booking between 3:00 p.m. and 4:30 p.m. on Friday, _____, 20___. After pre-booking, the Defendant shall be released, but the Defendant must return to the Prairie Hills Work Release Center the following morning, Saturday, _____, 20___, by 7:00 a.m. to begin serving the sentence. If successful in the program, the Defendant will be released at 5:00 p.m. Sunday

____ The Defendant is given credit for _____ days already served.

____ The Defendant's term of incarceration shall be served concurrently/consecutively to the term of incarceration in _____.

____ The Defendant will be granted work release under the terms and conditions of the Woodbury County Jail. Hours of work release are as follows:

_____

_____

____ In lieu of the jail sentence imposed in this Sentencing Order, the Defendant may serve _____ days on electronic monitoring. To qualify for electronic monitoring, the Defendant shall telephone the Woodbury County Jail (712-279-6040) no later than 20 days from the date of this Sentencing Order to arrange for electronic monitoring. If the Defendant fails to telephone the Woodbury County Jail as ordered, the Defendant forfeits the privilege of electronic monitoring and the sentence of incarceration will take effect. Mittimus shall issue _____, 20___, at _____ ___.m.

____ The Defendant will be granted release to attend the Batterer's Education Program as follows: _____

____ The Defendant will be granted release to attend Substance Abuse Treatment as follows: _____

____ The Defendant shall successfully complete a course for drinking drivers.

____ The Defendant shall pay restitution through the Clerk of Court to the following victims in the following amounts:

_____

_____

The restitution shall be paid on or before: _____

X The Defendant is fined $ 500 plus $ 150 surcharge. The fine and surcharge ~~shall be paid on or before:~~ is suspended., ~~20___. The Defendant is notified that if the Defendant was granted time to pay the fine,~~ should the Defendant fail to pay the ~~fine and costs~~ by the date specified, the Clerk of Court shall forward ~~this matter~~ to the Central Collections Unit for collection. This will ~~result in~~ an additional 10% charge being imposed on any unpaid balance.

____ As an alternative to paying the fine, the Defendant is ordered to perform _____ hours of community service work within _____ months of sentencing. An assignment fee of $25.00 must be paid to The Center if The Center's services are used for placement.

X The Defendant is placed on probation to the Third Judicial District Department of Correctional Services, 515 Water Street, Sioux City, Iowa 51103, telephone

number 712-252-0590, for a period of _____ year(s). The Defendant shall pay a probation enrollment fee in the amount of $250.00.

____ The Defendant shall obtain a substance abuse evaluation within 30 days of sentencing and will comply will all recommendations thereof for a period of twelve months.

____ The Defendant shall enroll in and successfully complete the Batterer's Education Program through the Third Judicial District Department of Correctional Services.

____ The Iowa Department of Transportation is ordered to revoke the Defendant's driver's license or driving privilege for 180 days pursuant to Iowa Code section 901.5(10).

____ The Defendant is ordered not to harass, intimidate or interfere with _____. The Defendant shall not go within 500 fee of this person, except during court appearances, or go within 500 feet of the residence, school or place of employment of this person. The Defendant shall not communicate in person, by telephone, or in writing with this person, or attempt to speak to or communicate with this person through a third party, except through the Defendant's attorney or a mutually agreed upon third person concerning any court ordered visitation or custody issues that may be in effect. This order shall remain in effect for one year from the date of sentencing.

____ The no contact order issued to the Defendant in this case was lifted on the _____ day of _____, 20____.

____ The court finds, pursuant to Iowa Code section 690.2, that the Defendant has not been fingerprinted. The Defendant is ordered to report to the Woodbury County Jail on or before the _____ day of _____, 20____, for the purpose of being fingerprinted and photographed. The Clerk of Court shall provide the Woodbury County Jail with a copy of this order regarding the fingerprinting and photographing of the Defendant. The Woodbury County Jail shall notify the Clerk of Court if the Defendant fails to comply with this order. If the Defendant fails to comply with this order, the Defendant may be held in contempt of court and a warrant shall issue for the arrest of the Defendant. Contempt of court is punishable by incarceration of up to six months and a fine not to exceed $500.00. The Clerk of Court shall return this file to the court if the Defendant has failed to comply with the order to be fingerprinted and photographed by the date listed above.

X See attached terms of probation

The sentence imposed in this case is based on the facts shown to the court, the plea agreement, presentence investigation and/or for reasons of deterrence. Other reasons include: _____

Costs are taxed to the Defendant. The Defendant was advised of the Defendant's right to a fifteen day delay before sentencing and of the Defendant's right to file a motion in arrest of judgment. The Defendant was advised that a criminal conviction, deferred judgment, or deferred sentence may affect the Defendant's status under federal immigration laws. Appeal bond is set in the amount of $ 2,500.

If the Defendant was represented by court-appointed counsel, the Defendant must pay restitution for attorney fees pursuant to Iowa Code section 815.9 for any costs incurred, and judgment is ordered for the same.

The Clerk of Court shall send a copy of this order to the attorneys of record and to the Defendant.

Judge, 3rd Judicial District of Iowa

Approved:

_____
Assistant Woodbury County Attorney

_____
Attorney for the Defendant

_____
Defendant

COPIES SENT TO:
✓ Co. Atty.
✓ Deft's Atty.
✓ Deft. c/o WCJail
✓ DOS
✓ Phoenix Project
6-30-04

cc: Jail 6-30-04
(CREIRTF + Prof. Phoenix Sealed Cpl. mass)

SPECIAL CONDITIONS OF PROBATION

Addendum

State of Iowa vs. Troy Kunkel

In addition to the standard rules and conditions of probation the following special conditions shall be a requirement of the defendant's probation:

- X Defendant shall pay the probation enrollment fee.
- ____ Defendant shall be subject to intensive probation with electronic monitoring to insure compliance. Defendant shall pay any and all costs.
- X Defendant shall obtain a substance abuse and/or mental health evaluation and follow all recommendations during supervision.
- ____ Defendant shall complete the Cognitive (victim) Empathy course.
- X Defendant shall complete the Anger Management training.
- ____ Defendant shall enroll and successfully complete the Batterer's Education Program.
- ____ Defendant shall attend and complete the Violator Program.
- ____ Defendant shall attend and complete the Treatment Alternatives to Street Crimes Program.
- X Defendant shall support the their dependents and meet other family responsibilities.
- X Defendant shall work regularly at least 30 hours at a lawful occupation unless excused by probation officer for schooling, training, or other acceptable reasons.
- X Defendant shall not frequent places where controlled substances are illegally sold, used, distributed, or administered.
- ____ Defendant shall not be at or enter any gambling establishments unless approved by their probation officer.
- ____ Defendant shall not frequent places that primarily serve alcohol/beer.
- ____ Defendant shall not associate with any persons engaged in criminal activity, and shall not associate with any person convicted of a felony unless granted permission to do so by the probation officer.
- ____ Defendant shall notify third parties by letter of risks that may be present by the defendant's criminal record or personal history. Defendant shall permit the probation officer to make such notifications and confirm the defendant's compliance with such notification(s).
- ____ Defendant shall write and mail letters of apology to the victim(s).
- X Defendant shall notify the probation officer of any material change in the defendant's economic circumstances that would prohibit the defendant's ability to pay any unpaid restitution, fines, child support, or any other obligations until such obligations are paid in full.
- ____ Defendant shall cooperate and comply with any orders entered by a United States District Court Judge or a United States Magistrate Judge regarding deportation.
- X Defendant shall reside in a community treatment center, halfway house,

Residential Treatment Facility or similar facility.

X Defendant shall remain at county jail pending a vacancy.

___ Defendant shall reside at a county jail with / without a work release.

~~SD~~ ___ Defendant shall be released, with supervision by probation officer, pending a vacancy.

X This requirement may be waived SOLELY at the probation officers discretion.

___ Defendant shall be subject to home detention from _____

_____

_____

X Defendant shall perform 20 hours per week of community service at a place approved by probation officer and provide proof of completion. If not employed by 10-11-2004

___ Defendant shall be restricted to their place of residence during evening and nighttime hours as necessary to protect the public from crimes that the defendant might commit during those hours. Electronic monitoring may be used to insure compliance. Defendant to pay all costs.

___ A defendant convicted of a sexual offense shall report the address where the defendant will reside and any subsequent change of residence to the probation officer, and defendant shall register as a sex offender in any state where the defendant resides, is employed, or is a student thereof.

___ Defendant shall participate in Project Compass if deemed eligible.

___ Defendant shall sign a wage assignment of not less than 10% of take home pay to pay restitution and court costs.

___ Defendant shall be granted work release upon verification by the county jail and subject to their rules and conditions.

___ Defendant shall have work search on _____ from _____ to _____.

___ Defendant shall obtain an evaluation and follow through with any and all sex offender treatment during supervision including consent to periodic polygraph tests to insure compliance.

X Defendant shall participate in any other programs deemed necessary for my rehabilitation by my probation officer.

___ Defendant shall submit his/her person, property, place of residence, vehicle, personal effects, to search at any time, with or without a search warrant, with or without a warrant of arrest or reasonable cause by any probation officer or law enforcement official.

Owner - Deborah Kunkel

X Defendant shall reside with his parents unless ~~exempted~~ granted permission by his probation officer.

___

X Enroll and successfully complete Project Phoenix

___

EXHIBIT O

IN THE IOWA DISTRICT COURT FOR WOODBURY COUNTY

THE STATE OF IOWA, Plaintiff, vs. TROY KUNKEL Defendant.	FILED '04 JUL 13 AM 11:27 CRAIG JENSEN CLERK OF DISTRICT COURT BY: CLERK DESIGNEE CRIMINAL NO. SRCR062687 ORDER NUNC PRO TUNC

On July 12, 2004 this file is presented to the court due to the omission of the length of probation the defendant was to serve.

The Court has reviewed its notes and consulted with the attorneys of record.

IT IS ORDERED NUNC PRO TUNC:

1. The defendant shall be placed on probation for **2 (two) years**.

2. That in all other respects the sentencing order and ruling filed on June 30, 2004 remains unchanged.

SO ORDERED

DUANE E. HOFFMEYER, Judge of the
Third Judicial District of Iowa

cc: CoA
P.Def
DCS
7-13-04sh